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A COMPARISON OF FUSARIUM AVENACEUM, F. OXYSPORUM, AND F. SOLANI VAR. EUMARTII IN RELATION TO POTATO WILT IN WISCONSIN[1]

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INTRODUCTION

The fusarium wilts of potato (*Solanum tuberosum* L.), while of relatively minor importance to the potato industry as a whole, constitute a major problem in many local areas. Their endemic occurrence in certain regions and their sporadic appearance in others render them constant hazards to the potato crop. Appel (*1*),[2] working in Germany in 1907, described a leaf-rolling disease of potato which he believed to be due to one or more species of *Fusarium*. The disease characteristically produced a folding together of the margins of the leaflets, while the lower leaf surfaces of diseased plants presented a leaden appearance and the entire foliage frequently assumed a reddish tint. The potato wilt diseases associated with *Fusarium solani* (Mart.) App. & Wr. var. *eumartii* (Carp.) Wr. and *Fusarium oxysporum* Schlecht. *f.1* Wr. (*F. oxysporum* f. *tuberosi* S. & H.) (*19*) have been studied extensively and the literature on the subject reviewed fully by Goss (*5*, *6*, *7*, *8*, *9*, *10*).

A potato disease differing somewhat in symptoms from the common potato wilts has been observed in Wisconsin for several years. From infected plants collected in the summer of 1936 a species of *Fusarium* was isolated which differed in cultural characters and spore type from *F. solani* var. *eumartii* and *F. oxysporum*. This culture was identified as *Fusarium avenaceum* (Fr.) Sacc. by Dr. Otto A. Reinking of the New York Agricultural Experiment Station. Several phases of the disease have been studied and in each case comparison has been made with the two wilt diseases mentioned above. For convenience, the three diseases will be referred to herein as eumartii wilt, oxysporum wilt, and avenaceum wilt.

PATHOGENICITY STUDIES

Many isolations of *Fusarium avenaceum* and various other species of *Fusarium* were made from diseased plants collected in commercial fields of central and northern Wisconsin during the summer of 1936. Single-spore cultures were obtained by the dilution-plate method on clear agar. Four plants each of Katahdin and Bliss Triumph varieties were inoculated with each culture. The fungus was introduced

[1] Received for publication December 2, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 524.

by inserting mycelium into a cleft cut into the stem just below the ground level after the stem surface had been disinfected. Sterile nonabsorbent cotton was placed over the wound and the soil was replaced. Isolates which produced a progressive discoloration of the vascular elements some distance from the point of inoculation were retained for further study; the remainder was discarded. Repeated tests showed *F. avenaceum* to be parasitic on potato, while plants inoculated with other fungus isolates and the control plants remained healthy.

In the belief that a pathogen new to potato had been obtained a soil inoculation test was set up with *F. avenaceum*. A mixture of 3 parts of composted soil to 1 part sand was autoclaved for 2½ hours, along with the 6-inch clay-pot containers. After exposure to the air for one week the autoclaved soil was inoculated with 23-day-old corn-meal-sand cultures of *F. oxysporum*,[3] *F. avenaceum*, and *F. solani* var. *eumartii*.[3] Suberized seed pieces of Irish Cobbler were planted and five pots of each inoculation were placed at air temperatures of 18° and 24° C.

Plants infected from the soil by *F. solani* var. *eumartii* were dead in 7 to 10 days after emergence at 24° and in 14 to 20 days at 18°. The plants infected by *F. avenaceum* or *F. oxysporum* were less seriously affected, living on an average for 65 days after emergence. The plants affected by oxysporum wilt displayed symptoms of leaf yellowing for an average of 20 days before complete wilt and death occurred. Those affected by avenaceum wilt showed early, distinct signs of the disease 20 days after emergence; the plants recovered for a time but finally succumbed to the wilt at the same time as did those infected by *F. oxysporum*. The control plants in sterilized soil to which sterile corn meal and sand had been added were healthy 80 days after emergence, at which time the experiment was discontinued. The respective organisms were reisolated successfully in all instances from the lower stem, roots, or tubers of infected plants. These results indicated that the strain of *F. avenaceum* tested was capable of producing infection and discoloration of the stems, stolons, roots, and tubers from which the fungus could be reisolated.

SINGLE-SPORE STRAINS

The pathogenicity of 12 single-spore strains of *Fusarium avenaceum* from one of the original isolates was tested by stem-puncture inoculation and by the use of inoculated, sterilized soil. In the artificially infested soil several weak strains were able to cause root infection and browning, but were not capable of affecting the vascular tissues of the stem to any great extent. When these cultures were injected into cleft wounds in the stem, a vascular discoloration extending 1 to 1½ cm. above the point of inoculation was produced after 20 days. No foliage symptoms were produced. Another group attacked the roots of plants in infested soil, invaded the lower stem, and produced in it a vascular discoloration extending 1 to 2 cm. above the point at which the infected roots were attached. Stem inoculations resulted in vascular penetration 2 to 3 cm. from the point of inoculation

[3] Dr. R. W. Goss, University of Nebraska, kindly furnished subcultures of *F. oxysporum* and *F. solani* var. *eumartii* from isolates on which he reported in 1940 (*10*).

after 20 days. Mild foliage symptoms were detected on a few plants in this group. The third group of single-spore strains readily produced root infection and vascular infection of the stem from infested soil, and definite foliage symptoms were apparent. Vascular penetration extended 5 to 7 cm. above the point of inoculation by the stem-puncture method. Thus the 12 single-spore isolates tested produced three more or less distinct classes of infection as measured by the extent of root and stem penetration and the resulting foliage symptoms. Seven of the most virulent single-spore lines were combined for use in later inoculation studies.

SEED-PIECE INOCULATION

Several methods of seed-piece inoculation were tested with *Fusarium avenaceum*, *F. oxysporum*, and *F. solani* var. *eumartii* in the greenhouse. Fifty seed pieces each of Irish Cobbler and Rural New Yorker were used for each treatment and planted at once in flats of sterilized soil. Uninoculated controls for each method were included. The methods were as follows:

Presuberized.—The seed piece was cut and allowed to stand in a ventilated, saturated atmosphere for 4 days at 24° C. to allow suberization and cork formation to take place before inoculation; it was then dipped in a suspension of spores and mycelium of the fungus and planted.

Fresh cut.—Freshly cut seed tubers were dipped at once in the fungus suspension and planted immediately.

Plugged.—A plug was removed from the seed piece; a disk of mycelium and agar was inserted; the plug was replaced and sealed with paraffin.

Cleft.—A ¼-inch cleft was cut near the eye of the seed piece, mycelium was inserted with a needle, and the cleft was resealed.

Plants inoculated with *F. avenaceum* and *F. solani* var. *eumartii*, together with the uninoculated controls, were held at 24°; *F. oxysporum* inoculations were held at 28°.

The measure of success for each method was determined by recording the amount and degree of seed-piece and stem infection after 22 days when the plants were 8 to 10 inches high. Percentage of infection and disease-index values were calculated from these tabulations. The disease index is similar to that used by Walker, Larson, and Albert (*20*) in the analysis of potato scab data.[4] The cleft and plugged methods gave severe infection when *F. solani* var. *eumartii* was used and numerous plants failed to emerge or were killed soon after emergence. *F. avenaceum* and *F. oxysporum* failed to give consistent infection by these methods under greenhouse conditions. The results for the presuberized and fresh-cut methods are shown in table 1. Although the numbers were too small for satisfactory statistical treatment, there were definite differences between species, varieties, and the two methods of inoculation.

[4] The method of calculation was as follows: Group-weight values were given: for clean—0, for slight infection—1, for moderate—2, and for severe infection—5. These arbitrary values were allocated to represent the relative severity of the respective grades. The number of seed pieces or stems in each class was multiplied by its respective class weight; the products were then added and the total multiplied by 100. The figure obtained was divided by five times the total for the treatment to arrive at the disease index for that treatment, i. e., $\frac{\text{total of weighted values} \times 100}{\text{total for treatment} \times 5}$ = disease index. Disease index is, therefore, a combined measure which expresses in one value both the percentage and the degree of infection.

TABLE 1.—*The effect of two methods of inoculation with three species of Fusarium on seed-piece and stem infection in the greenhouse*

[Each figure represents 50 potato seed pieces]

Potato variety and species of *Fusarium*	Seed-piece infection				Stem infection			
	Presuberized		Fresh cut		Presuberized		Fresh cut	
	Percent	*Disease index*	*Percent*	*Disease index*	*Percent*	*Disease index*	*Percent*	*Disease index*
Irish Cobbler:								
F. solani var. *eumartii*	20.0	7.6	84.0	29.6	9.1	5.4	37.4	19.2
F. avenaceum	21.0	7.2	24.0	11.2	6.9	3.0	14.6	4.4
F. oxysporum	20.0	15.8	38.2	23.6	5.5	1.6	27.1	10.6
Uninoculated control	2.0	.4	4.2	.8	0	0	0	0
Rural New Yorker:								
F. solani var. *eumartii*	100.0	78.0	100.0	100.0	64.5	27.4	78.0	63.0
F. avenaceum	14.0	5.6	32.0	12.0	6.0	1.2	14.3	3.0
F. oxysporum	44.0	14.4	76.0	55.0	21.4	4.7	72.0	20.0
Uninoculated control	0	0	2.0	.4	0	0	0	0

A beneficial effect of suberization was seen on both potato varieties, although Rural New Yorker thus treated was more severely attacked than Irish Cobbler. The effect of suberization was evidenced by a decrease in severity, or by a delay in attack, together with a reduction in the number of plants affected. The "fresh-cut" inoculations generally allowed a greater amount and a greater degree of infection than did the presuberized treatment.

F. avenaceum and *F. oxysporum* produced consistent infection of seed pieces and stems, but both species were in general less infectious than *F. solani* var. *eumartii*. The most extensive and most severe infection was found with *F. solani* var. *eumartii* on Rural New Yorker variety, where the disease index more nearly approached the percentage values than was the case with the other species. A close relationship was noted between seed-piece rotting and stem infection. Reisolations from the stems of each lot yielded the respective organism used in inoculation.

These results are in general agreement with those of Goss (*5*, *6*) with *F. oxysporum* and *F. solani* var. *eumartii* and those of MacMillan (*13*) with *F. oxysporum*. The differences found between the three fungus species in the greenhouse experiments are consistent with the results observed later in field inoculations, although the same differences between the two varieties and the two methods of inoculation were not found in the subsequent field experiments.

SYMPTOMS OF AVENACEUM WILT

The first symptom to appear on the foliage following infection by *Fusarium avenaceum* is a faint yellowing of the basal portions of the leaflets of the apical leaves. This stage is soon followed by more pronounced yellowing, approaching chlorosis, of the leaflets, together with a bunching of the leaflets and folioles due to a shortening of the internodes of the petiole. The apical leaves are reduced in size and there is a slight increase, under greenhouse conditions, in the number of leaflets and folioles. The leaves frequently assume a greyish cast and the texture becomes brittle and coarse as compared with that of the normal foliage.

The stages which follow vary to a large extent with different environmental conditions. With sufficient soil moisture and cool temperature,

the affected plant continues to grow, and carbohydrates accumulate in the above-ground organs. Characteristic red or purple pigmentation occurs, together with an upward rolling of the leaves (fig. 1) and the production of aerial tubers in the leaf axils (figs. 2, 3). The plant at this stage resembles one suffering from rhizoctonia stem injury, while other combinations of stunting, yellowing, leaf rolling, and the presence of aerial tubers may produce a plant somewhat similar in appearance to haywire (*9*) of psyllid yellows (*17*). To distinguish between these diseases and avenaceum wilt on the basis of foliage symptoms alone is difficult; in this group, however, only avenaceum wilt consistently produces vascular discoloration of the lower portion of the infected stem.

FIGURE 1.—Symptoms of avenaceum wilt showing the effect of inoculation with *Fusarium avenaceum* on Bliss Triumph potato at Madison, Wis. Note the upward rolling of the leaves and wilting of the plant.

When low soil moisture and unfavorable high temperatures prevail, rapid wilting and death of the affected plant result sometimes, preceded by a gradual loss of chlorophyll and consequent yellowing of the foliage. Tipburn and loss of the lower leaves are common. Plants infected with *F. avenaceum*, whether grown in the greenhouse or field, are frequently indistinguishable from those affected with oxysporum wilt. This is particularly true where temperature and water supply are constant, or when high temperatures prevail. Inoculations in the field with *F. avenaceum* at Madison, Wis., occasionally produced slight red and purple coloration of the foliage accompanied by rolling of the leaves, but no aerial tubers were formed (fig. 1). In no case were these plants as striking in appearance as infected plants in a similar inoculation plot 200 miles north of Madison (figs. 2, 3) where

purpling of the foliage and formation of aerial tubers were much more prevalent. Plants in this location affected with oxysporum wilt, and

FIGURE 2.—Symptoms of avenaceum wilt showing the effect of inoculation with *Fusarium avenaceum* on Bliss Triumph potato at Starks, Wis. Note the upward rolling of the leaves and the axillary aerial tubers.

a few with eumartii wilt, displayed symptoms similar to those produced by *F. avenaceum*, but most of those affected with eumartii wilt

displayed traces of the interveinal mottle and bronzing characteristic of that disease.

The symptoms of avenaceum wilt described above are those most commonly found associated with mid- to late-season field infection in

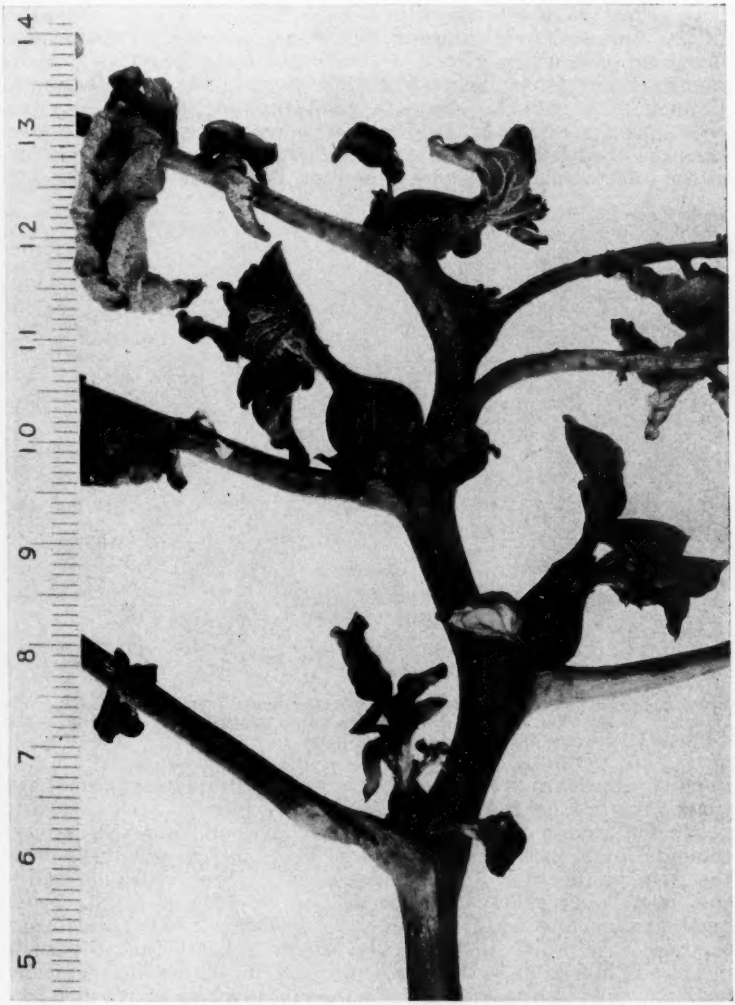

FIGURE 3.—The formation of aerial tubers in the leaf axils of a purple top potato plant infected by *Fusarium avenaceum* from the plot at Starks, Wis.

the northern half of Wisconsin. Severe early infection has been observed under field conditions, and especially after artificial inoculation. Plants affected thus either recover for a time, meanwhile showing partial masking of symptoms, or they succumb soon after emergence

with a consequent reduction in stand and yield. Plants which are severely infected early in the season show stunting and bunching of the tops and a decrease in chlorophyll at the apex of the plant strikingly similar to yellow dwarf. However, infection by *F. avenaceum* does not result in discoloration of the pith like that in plants affected with yellow dwarf or eumartii wilt.

The roots and stems infected with *F. avenaceum* are indistinguishable from those affected by other fusarium wilts of the potato. Infection may develop unilaterally in the stem, or may be distributed throughout all vascular elements. Browning of the latter may precede fungal invasion or the two may be coincident. Discoloration has been observed to extend 6 to 8 inches above the soil level in full-grown infected plants, although the causal organism has seldom been

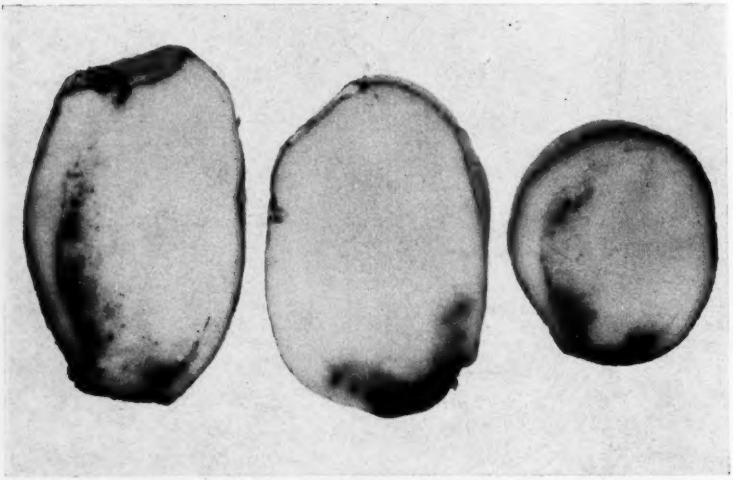

FIGURE 4.—Severely infected tubers from potato plants inoculated with *Fusarium avenaceum* showing vascular discoloration and necrotic spots in the cortex and pith. Photograph taken at the time of harvest.

isolated 3 inches above the soil. Pionnotes of the fungus were sometimes observed on the exterior of severely infected stems, slightly above the ground level. Actual fungal invasion, however, is more common in the below-ground parts. The infection progresses from the stem to the tuber through the stolon. Direct infection of the stolons or young tubers from the soil has not been observed. Seed-piece infection may occur, but the late-season effects of the disease and the ease with which root infection occurs indicate that the latter method of infection is the more common in Wisconsin. Vascular browning can likewise be traced macroscopically from an infected root into the stem xylem.

Affected tubers show a vascular discoloration which originates at the point of stolon attachment and extends into the tubers (fig. 4), varying among plants and among the tubers of an individual plant. Severely infected tubers may show a dry stem-end rot (fig. 5), which

is externally discernible as a sunken area at the basal end of the tuber. This symptom differs from the water-soaked appearance of tubers affected by *F. solani* var. *eumartii* in that a characteristic powdery dry-rot is produced. The affected parts are gray to pink in color, showing occasional traces of bright red. The mycelium of the fungus is readily apparent, and reisolation is easily accomplished from such specimens. Although tuber discoloration is typically vascular, symptoms in pith and cortex have been observed in certain experimental lots, consisting of hard, corky, dark-brown, necrotic areas scattered in the flesh of the tuber (fig. 4).

The first sign of oxysporum wilt under Wisconsin conditions is a yellowing of the lower leaves, which gradually spreads to the leaves directly above. Wilt and death may follow rapidly, or the stem may

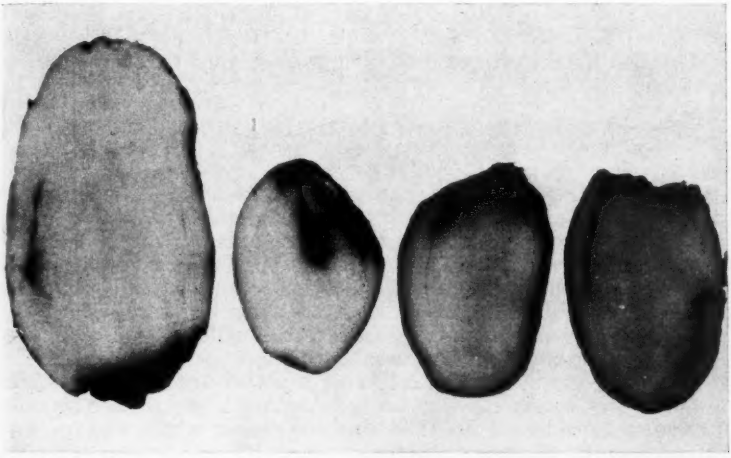

FIGURE 5.—Severely infected tubers from potato plants inoculated with *Fusarium avenaceum* showing vascular discoloration and a dry stem-end rot. Photograph taken at the time of harvest.

remain turgid until most of the plant has a golden-yellow cast, with only a few green leaves remaining at the tip. The appearance of the roots, stems, and tubers of infected plants is similar to that described by other workers (*2*, *5*, *8*, *13*, *18*), and these below-ground symptoms are frequently indistinguishable from those of the other fusarium wilts.

The symptoms of eumartii wilt in Wisconsin are similar to those described by Goss (*6*, *8*), and infection is readily secured under a wide range of conditions in the greenhouse or field. Rural New Yorker variety is an exception, frequently showing only the irregular, interveinal bronze areas on the leaflets without interveinal chlorosis. Tip burning and dropping of the leaves occasionally occur independently of any other symptoms. Russet Rural potatoes in commercial fields [5] show this same phenomenon, and similar symptoms have been observed by the senior author on Bliss Triumph potatoes growing in western Nebraska. These plants previously had been

[5] So far as the writers know, this is the first report of eumartii wilt in Wisconsin.

retarded by dry weather, so that severe tip burning on the leaves of affected plants was the only apparent sign of the disease on the above-ground parts. Severe seed-piece infection and early death of the plant have been observed only when a large quantity of inoculum was present.

Leach and Darling [6] in 1935 reported similar symptoms in Minnesota on plants affected by unidentified species of fusaria. In 1939 Leach (*12*) further stated that the wilt caused by the tarnished plant bug was not identical with the purple top wilt, which disease he indicated might be due to the aster yellows virus. Decker [7] reported western aster yellows on potatoes in New York which agreed closely with the descriptions of purple top wilt and the blue stem disease described by Orton and Hill (*14*). Hill (*11*), after examining potato tubers affected with blue stem, purple top wilt, net necrosis, stem end browning, and avenaceum wilt, reported differences in the location of suberized deposits and wound cork, but found no microchemical differences in the wound periderm among the different types of material.

DISTRIBUTION AND ECONOMIC IMPORTANCE OF FUSARIUM WILTS IN WISCONSIN

Avenaceum wilt varies in severity from year to year, and, like oxysporum wilt, is influenced by environmental factors and by the vigor of the host plant. Leaf rolling, reddening of the foliage, and formation of aerial tubers are symptoms which occur chiefly in the northern half of the State, and they appear most prominently after mid-August. For this reason, the late-maturing varieties usually sho a higher percentage of infected plants under natural field conditions than do the early varieties. Lower temperatures accompanied by heavy or frequent rains, following a period of drought and high temperatures, commonly lead to the advent of this symptom complex 1 week or more later. In Wisconsin the disease appears to be more prevalent in the light-textured soils.

The resemblance of avenaceum wilt to some other potato disorders and its sporadic appearance make estimation of its economic significance difficult. The amount of infection is generally small, involving from 2 to 5 percent of the plants in a field, although fields showing up to 20 percent infection have been observed, and small areas within a fie'd may show much higher percentages of plants with foliage symptoms than are present in the field as a whole. It is believed, however, that the diseases of *Fusarium oxysporum* and *F. avenaceum* are equal in importance and that these two species are largely responsible for the fungus wilts of potatoes in Wisconsin.

F. solani var. *eumartii* has been isolated from field and bin specimens in this State, and fields showing 8 percent of the plants affected by this organism have been observed. Eumartii wilt, however, is confined largely to the northeast section of the State and even there it is not the serious problem to potato growers that it is in some other parts of the country.

[6] LEACH, J. G., and DARLING, HENRY. SYMPTOMS OF FUSARIUM WILT OF POTATO IN MINNESOTA THIS YEAR. U. S. Bur. Plant Indus., Plant Dis. Rptr. 19: 299–302. 1935. [Mimeographed.]

[7] DECKER, PHARES. A NEW POTATO DISEASE IN NEW YORK. U. S. Bur. Plant Indus., Plant Dis. Rptr. 23: 226–227. 1939. [Mimeographed.]

RELATION OF THE PARASITE TO THE HOST TISSUE

To observe the path of the fungus in the plant, potato seed pieces were planted in sterilized soil in glass containers, which were covered to exclude light. The soil was moistened with sterilized, distilled water through a tube at one side of the container. A suspension of spores was introduced through the watering tube when the roots had accumulated around the end of the tube. Daily observations were made with a dissecting microscope and plant parts were removed at intervals and prepared for sectioning. *Fusarium avenaceum*, *F. oxysporum*, and *F. solani* var. *eumartii* were studied comparatively in this manner.

Water soaking of the root tips appeared at 2 days with *F. solani* var. *eumartii*, whereas it did not become definite with *F. avenaceum* and *F. oxysporum* until the seventh day. The hyphae of *F. solani* var. *eumartii* were apparent in the cells of the epidermis and cortex of certain rootlets 7 days after inoculation. In the more severely affected rootlets the walls of the cortical cells and of the younger xylem cells were broken down. The epidermal cells were thus separated from the larger cells of the stele but still both groups remained in their original positions. The later stages of invasion with *F. solani* var. *eumartii* were similar to those illustrated by Goss (*6*). The cortex of the older roots became permeated with the intracellular hyphae of the fungus, which eventually reached the xylem. Mycelia occurred abundantly in the cortical cells of the lower stem and were also found in the spiral and pitted vessels at the junction of the infected stem and roots. Some of the smaller pitted vessels of the xylem in the stem collapsed, and the thinner, weaker-appearing cell walls indicated the effect of solvent action; in others, which were still thick-walled, dense granular deposits were found. Necrosis of cell groups in the inner and outer phloem, together with collapse of the xylem, occurred, in advance of the fungus hyphae.

In the early stages of penetration by *F. avenaceum* and *F. oxysporum*, mycelia were found in the epidermal and cortical cells of the young rootlets. The cell walls appeared to have become softened and swollen and some cells were partially separated from the adjoining ones. This dissolving action was not so rapid as that observed with infection by *F. solani* var. *eumartii*. *F. avenaceum* was found commonly in the xylem vessels of roots and stems, and hyphal branches radiated from the infected xylem vessels into the outer phloem of the stem where collapse and necrosis of the cells occurred. Many of the thick-walled xylem cells of the stem became plugged. The cortex of the lower stem was also invaded. In the younger roots infected by *F. oxysporum*, mycelia were found in the xylem vessels of the root, as well as in the cortex. In larger roots and in the lower stem the hyphae occurred chiefly in the xylem.

It may be seen that the three fusaria related themselves to the potato in a very similar manner. *F. solani* var. *eumartii* was the most active cortical invader, and enzymic action in advance of the pathogen was greatest. *F. avenaceum* invaded the cortex of root and stem, but was more aggressive than the last-mentioned fungus as a vascular invader. *F. oxysporum* was about equal to *F. avenaceum* as a vascular parasite, but it was less conspicuous in the stem cortex.

TEMPERATURE AND MOISTURE RELATIONS

In order to determine the effect of natural environment on the production and development of wilt diseases, it is necessary to understand the role of temperature and moisture in infection. Therefore, studies of the effect of temperature on the growth of the avenaceum

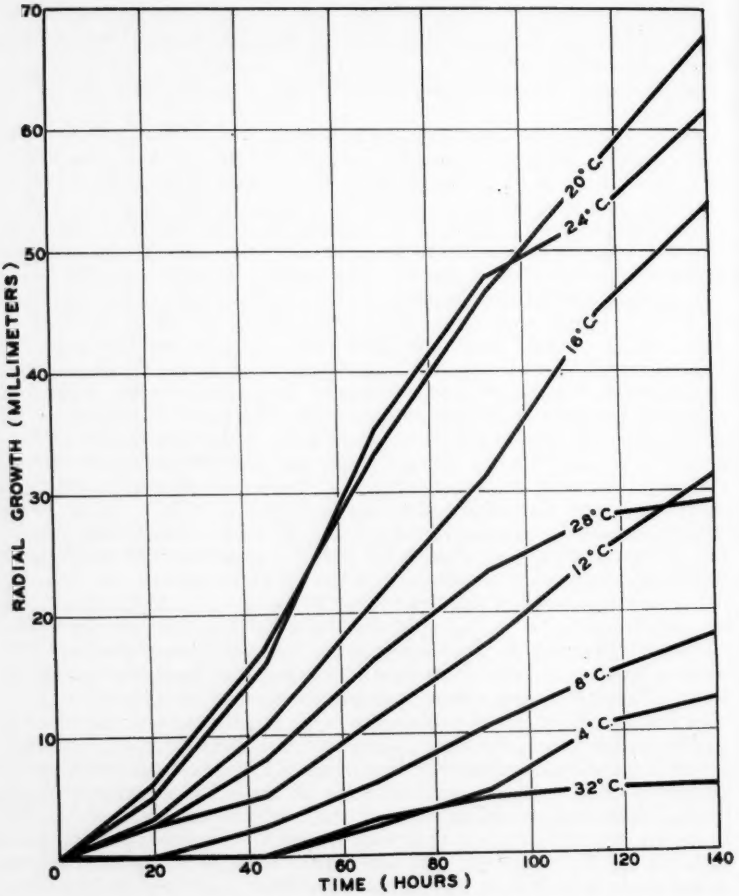

FIGURE 6.—The effect of temperature on the growth of *Fusarium avenaceum* on potato-dextrose agar.

wilt fungus, together with the effect of soil temperature and moisture on development of the disease, were undertaken.

RELATION OF TEMPERATURE TO GROWTH OF THE FUNGI

Plates of potato-dextrose agar adjusted to neutral reaction were inoculated with 4-mm. disks taken from the periphery of a 5-day-old

actively growing Petri-dish culture of *Fusarium avenaceum*. Five replicates were placed at each of eight temperatures ranging at 4° intervals from 4° to 32° C., inclusive. Increments of radial growth were measured daily.

The results are shown graphically in figure 6. Both daily-increment and total-growth measurements exhibited the greatest response at temperatures of 20° and 24° C. These results agree rather closely with those of Cormack (*4*), who reported an optimum of 24° for a strain of *F. avenaceum* isolated from alfalfa. The straight-line trends in this graph indicate that growth was not retarded by the accumulation of staling products or other byproducts of the fungus. The optimum is lower than the optima reported for *F. solani* var. *eumartii* (25°) (*6*) and *F. oxysporum* (30°) (*7*) although Goss (*5*) demonstrated that the optimum temperature for growth of the latter varied with the substrate.

EFFECT OF SOIL MOISTURE AND SOIL TEMPERATURE

Wisconsin soil-temperature tanks were employed in the determination of the effect of soil temperature and moisture on infection and on growth of uninoculated plants. Temperatures were automatically controlled to ±0.5° C. at each of the following temperature levels: 16°, 20°, 24°, 28°, and 32°. The air temperature fluctuated between 20° and 25°. A mixture of one-half sand and one-half composted soil in burlap bags was autoclaved at 20 pounds steam pressure for 2½ hours. Inoculations were made several days before planting, after the soil had been exposed to the air for 10 days. The sand and corn-meal inoculum used in the first series was abandoned in favor of autoclaved barley kernels in later trials. The Katahdin and Irish Cobbler varieties were planted in the first two series, Rural New Yorker in subsequent tests. Four presuberized seed pieces were planted in each can of autoclaved, artificially infested soil. Five separate series were completed. In the first two a comparison was made between plants inoculated with *F. avenaceum*, *F. solani* var. *eumartii*, and *F. oxysporum* and uninoculated controls. In the remaining three series only *F. avenaceum* was used. The soil moisture was maintained throughout at 40 and 60 percent of the moisture-holding capacity of the soil.

The best growth of the foliage of the potato plant was produced in the greenhouse at constant soil temperatures of 20° and 24° C. when the air temperature was maintained at 20° to 25°. As soil temperature increased, germination was hastened and leaf size decreased. At 28° the plants were decidedly smaller, while at 32° growth was slow and abnormal, resulting in poorly developed tops. Plants grown at a soil temperature of 16° developed more slowly than those at 20° and 24°, although thicker stems and larger leaves resulted and the plants ultimately produced more leaf area. Better germination and larger plants prevailed throughout the temperature series at 60 percent of the soil moisture-holding capacity. At 28° and 32° plant growth was retarded at 40 percent moisture, a result undoubtedly of the more rapid depletion of soil moisture by the greater rate of evaporation and transpiration at these temperatures. The lower the temperature, the less was the effect of moisture on plant size.

At the temperatures ranging from 20° to 28° C., the plants in inoculated soil were from 2 to 6 inches higher than the controls while the

reverse was true at 32°. At 16° no differences were observed. Root growth in all inoculated plants was correspondingly greater than in the controls. At the lower level of soil moisture a slight increase in the percentage of plants infected by *F. avenaceum* and *F. oxysporum* resulted at 16°, while a decrease occurred with *F. solani*, var. *eumartii* at 28° and with *F. oxysporum* and *F. avenaceum* at 32°, but the number of plants was too small to show a significant difference. The data for the two moisture levels at each temperature are combined in table 2.

TABLE 2.—*The effect of soil temperature on infection of potato plants by three species of Fusarium*

Soil temperature (° C.)	Symptoms in plants in soil inoculated with—			Symptoms in plants in uninoculated soil
	F. solani var. *eumartii*	*F. oxysporum*	*F. avenaceum*	
	Percent	*Percent*	*Percent*	*Percent*
16	75	10	12	0
20	100	10	43	0
24	100	70	62	0
28	93	100	100	8
32	[1]	[2] 92	[2] 80	[2] 0

[1] Mechanical difficulties. No infection at time of injury to plants.
[2] Represents 1 series only.

The effect of temperature on *F. oxysporum* and *F. solani* var. *eumartii* was similar to that observed by Goss (*5*, *6*), who reported an optimum of 30° C. for infection by the former and of 20° to 25° by the latter. The average percentage of infection by *F. avenaceum* for the five series at two levels of moisture showed a definite optimum at 28° for each moisture level (table 3), while it was consistently less at the lower moisture level at 16°. However, although the development of avenaceum wilt was more severe as the soil temperature increased up to the optimum, it was also more severe in the drier soil in spite of the fact that the percentage of infection was greater in the moister soil.

Two trials were made with Rural New Yorker to determine the effect of soil moisture on infection when the temperature remained constant. Soil was adjusted to 30 percent, 50 percent, and 70 percent of the moisture-holding capacity. Soil temperature was kept at 24° C. in the first series and at 28° in the second, while the average air temperature was 20° to 25° in both cases. Infection was greatest at the 50-percent level and least at 30-percent in both experiments (table 4).

The optimum temperature for growth of *F. avenaceum* is approximately the same as that required for rapid, sturdy development of the potato plant (20°–24° C.) while the optimum soil temperature for infection is some 4° higher. Thus although a difference of more than 5° exists between its growth optimum and that of *F. oxysporum*, they appear to have closely similar optimum temperatures for infection. Infection by *F. avenaceum* was more severe at 50 percent of the water-holding capacity of the soil than at either 30 or 70 percent. Similar behavior was reported for *F. oxysporum* (*5*), while soil moisture has relatively little influence on infection by *F. solani* var. *eumartii* (*6*).

TABLE 3.—*The effect of soil temperature on infection of potato plants by Fusarium avenaceum at two levels of soil moisture*

Soil temperature (° C.)	Symptoms in plants in soil inoculated with *F. avenaceum* at—		Symptoms in plants in uninoculated soil at—	
	60 percent of moisture-holding capacity [1]	40 percent of moisture-holding capacity [1]	60 percent of moisture-holding capacity [2]	40 percent of moisture-holding capacity [2]
	Percent	*Percent*	*Percent*	*Percent*
16	0	15	0	0
20	50	50	0	5
24	62	58	10	5
28	94	70	5	10
32	72	50	0	0

[1] Average of 5 replicates representing a total of 76 plants.
[2] Average of 5 replicates representing a total of 52 plants.

TABLE 4.—*The effect of soil moisture on infection of potato plants by Fusarium avenaceum at constant temperature*

Water-holding capacity of the soil (percent)	Symptoms in plants in soil inoculated with *F. avenaceum* [1]	Symptoms in plants in uninoculated soil [2]
	Percent	*Percent*
30	10.3	0
50	53.5	0
70	17.3	3.3

[1] Average of 2 experiments representing a total of 40 plants, 20 at 24° C. and 20 at 28° C.
[2] Average of 2 experiments representing a total of 24 plants, 12 at 24° C. and 12 at 28° C.

SEASONAL DEVELOPMENT OF THE DISEASE

The expression of symptoms of the fusarium wilts of potato present a constantly changing picture in commercial fields in Wisconsin. In the northern half of the State infected plants display varying stages of rosetting and upward curling of the leaves. The foliage parts are highly pigmented and the buds in the leaf axils are stimulated into the formation of aerial tubers. In the central and southern sections, infection usually results in wilting and rapid collapse of the plants. In still other more or less isolated regions of Wisconsin, the symptoms are very typical of those described for eumartii wilt (*6*). Disease development is undoubtedly influenced by the time and amount of infection and by the soil and climatic environments. The symptoms generally occur late in the growing season. This fact gives the impression that the potato varieties which are normally mature in late August are more resistant than the late varieties. However, Goss (*7*, *8*,) has observed that the amount of infection by *Fusarium* is influenced by date of planting as well as by variety.

In order to obtain a more adequate basis for the interpretation of the development of the wilt diseases throughout the season, the following experiments were carried out. Several varieties of potato were inoculated by various methods and planted on several dates in each of two widely separated locations in the State. These studies

were conducted during three successive seasons. Plants affected with avenaceum wilt, oxysporum wilt, and eumartii wilt were compared throughout with the uninoculated plants.

PLAN OF FIELD EXPERIMENTS

In 1937, a factorial experiment was conducted in the field at Madison, Wis., randomized blocks being used. Four potato varieties, three species of *Fusarium*, and three methods of inoculation were tested at each of three dates of planting with the result that potato varieties, fungus species, methods of inoculation, and dates of planting could be studied separately and in combination with each other. One-fourth of the total number of rows in the plot were uninoculated controls. Two standard early, maturing varieties, Irish Cobbler and Bliss Triumph, and two late-maturing varieties, Katahdin and Rural New Yorker, were used. Inoculation was made at the time of planting in all cases. The three methods of inoculation employed, namely, plugged, presuberized, and fresh-cut, have been described in the section dealing with inoculation methods. In several other tests, either corn-meal-sand inoculum or inoculated, sterilized soil was added to the seed piece in a paper bag and then planted. Plantings were made on May 25, June 9, and June 22, in order to include the range of the usual planting season for southern Wisconsin. Notes on the incidence and severity of foliage symptoms were taken at regular intervals throughout the growing season. Tuber discoloration was recorded for each individual lot after harvest on October 5.

The planting plan used in 1938 was similar to that of 1937. The three *Fusarium* species and four potato varieties were again included. Six replicated series were included, all planted on one date.

In order to study more fully the effect of environment on production of symptoms and on subsequent disease development, two locations were used in 1939. In addition to the plot at Madison, a planting was made at Starks, Wis., near Rhinelander, approximately 200 miles north of Madison. Planting in these two locations allowed for the study of the effect of two distinctly different environments on the development of the three wilt diseases. The plot at Madison was set up to study the effect of three methods of inoculation. Seed tubers of Irish Cobbler and Rural New Yorker were inoculated in three ways: By dipping freshly cut seed in fungus suspension, by using inoculated soil in paper bags along with the seed pieces, and by employing barley-kernel inoculum in the same manner. Uninoculated and inoculated rows were randomized within each of three replicates. The individual row size was increased to 50 plants for each inoculation as compared with 10 plants used in previous years. The purpose of the larger sample was to obtain greater uniformity and to determine whether any bias had resulted from the smaller test rows formerly employed.

In the plot at Starks six replicates each of Bliss Triumph and Rural New Yorker were planted. Inoculations were made in two ways: By dipping freshly cut tubers in a fungus suspension, and by the use of artificially infested soil. The plot was bordered on one side by grassland and on the other three sides by summer-fallowed ground, and was isolated from any other potato fields by a distance of one-half mile.

All experimental plots were sprayed frequently throughout each growing season with bordeaux mixture and calcium arsenate.

SEASONAL DEVELOPMENT OF SYMPTOMS ON THE FOLIAGE

The rate of development of top symptoms appeared to be closely correlated with the extent of infection, and to be favored by conditions conducive to rapid depletion of soil moisture. Wilt-disease readings

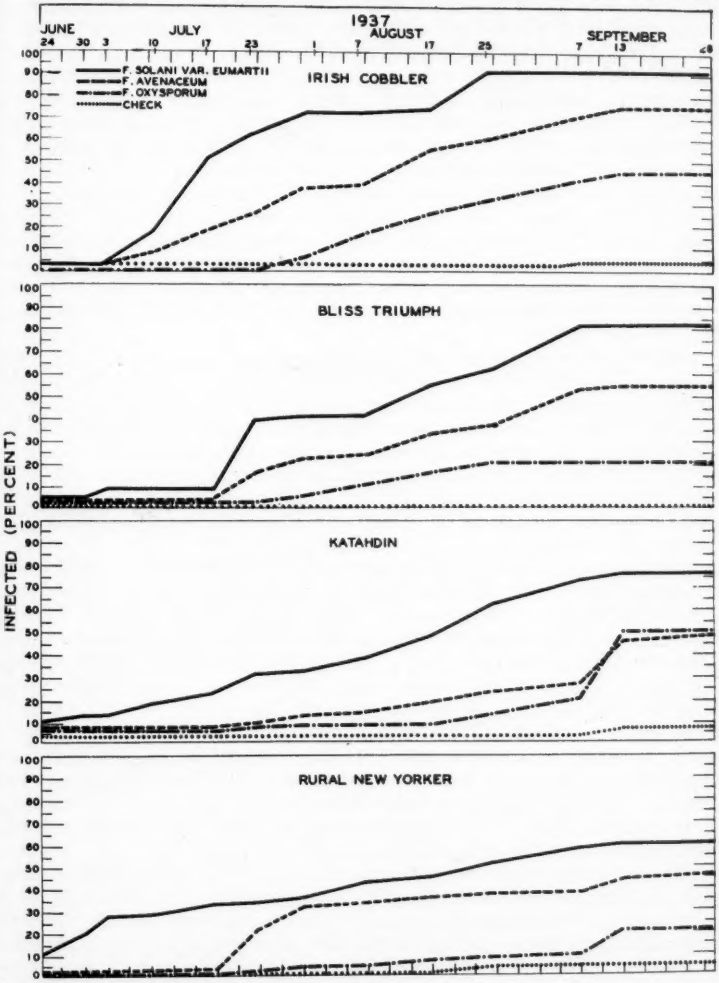

FIGURE 7.—The occurrence throughout the growing season of distinctive signs of the disease produced when four varieties of potato were inoculated with each of three wilt fusaria at Madison, Wis.

were recorded in the inoculated and uninoculated rows as soon as positive symptoms appeared. The percentages of inoculated and uninoculated plants showing symptoms on the foliage at various dates throughout the season for each of 4 potato varieties and 3

species of *Fusarium* in 1937, are shown graphically in figure 7. Thus each curve is based on the data from 90 plants. All plants which did not emerge were counted as infected and because of the irregular appearance of symptoms, the results from three dates of planting were combined. The time of appearance of symptoms varied with the individual host plant and with the species of *Fusarium* concerned. Some plants displayed definite signs of the disease soon after emergence, while others remained apparently healthy until the approach of maturity. Inoculation with *F. solani* var. *eumartii* produced the earliest symptoms and consistently showed the greatest percentages of infection, while *F. avenaceum* and *F. oxysporum* maintained a lower level of infection but caused a rapid rise in the number of plants showing symptoms late in the season. The symptoms of *F. avenaceum* were better defined, and therefore the curve for that species is higher than that of *F. oxysporum*. *F. solani* var. *eumartii* is clearly shown to be the most aggressive parasite as measured by both earliness of disease development and by the percentage of plants affected in all varieties. The percentage of diseased plants in the uninoculated rows was uniformly low.

Somewhat lower percentages of affected plants occurred with Bliss Triumph and Katahdin than with Irish Cobbler. These differences are consistent with those discussed later on tuber discoloration in the same varieties. The lower percentages of infection recorded for Rural New Yorker are explained in part by the fact that in this variety, as well as in Russet Rural, symptoms often consist only of tip burning and dropping of the leaves. These symptoms were not distinctive for any individual species of pathogen and were not recorded, therefore, as positive signs of the diseases concerned.

To demonstrate the validity of the graph in figure 7, a measure of the time required for the appearance of symptoms, that is, the average number of days to the appearance of symptoms was calculated, using the time elapsing between planting and the first appearance of symptoms for each plant and giving a value of "zero days" to all nonemerging plants and to plants which died within 1 week after emergence. Healthy plants, i. e., those showing no symptoms before maturity were given a value of 200 days. Low numbers, therefore, indicate early infection and symptom production, while higher numbers approaching 200 show that the plants were healthy throughout the season. If the foliage and tuber symptoms were closely correlated a statistical analysis of foliage symptoms would give results closely comparable to those obtained for the tuber symptoms.

The correlation between the time required for symptom appearance and the amount and severity of tuber symptoms is shown in table 5. A negative correlation ($r_2=-0.27$) was found between the days required for symptom expression and the percentages of tubers showing symptoms. For the correlation of days and disease index a significant correlation value was obtained ($r_1=-0.79$). The difference between the two correlations ($Z_2-Z_1=0.79\pm0.47$) was significant. These relationships show that days and index values were significantly more comparable than were days and percentage values. In other words, as the time required for primary-symptom expression increased, there was correspondingly less tuber infection and the severity of the latter decreased. It will be noticed that the inverse relationship for days

as compared with the amount and degree of tuber infection is maintained in general throughout tables 5, 7, and 8. Analysis of variance of

TABLE 5.—*Correlation of the time required for the production of foliage symptoms with the percentage and index of tuber discoloration at Madison, Wis., 1937* [1]

Species of *Fusarium* and potato variety	Average time required for symptom expression	Vascular discoloration of the tubers	
	Days	*Disease index*	*Percent*
F. avenaceum:			
Bliss Triumph	126	3	11
Irish Cobbler	96	10	31
Katahdin	142	5	19
Rural New Yorker	136	17	51
F. solani var. *eumartii:*			
Bliss Triumph	85	36	47
Irish Cobbler	64	53	70
Katahdin	92	36	55
Rural New Yorker	108	38	61
F. oxysporum:			
Bliss Triumph	171	3	12
Irish Cobbler	144	7	24
Katahdin	142	4	19
Rural New Yorker	176	12	43
Total	1,482	224	443

[1] r_1 of days and index = − 0.79; r_2 of days and percent = − 0.27. $Z_2 - Z_1 = 0.79 \pm 0.47$. At the 5-percent level of significance, for 10 degrees of freedom, $r = 0.58$.

the values for the number of days to symptom expression gave significant differences similar to those found when the data on tuber symptoms were analyzed.

The above correlations (table 5) were derived to ascertain the general association of foliage symptoms with the amount and severity of tuber symptoms. It cannot be concluded that these relationships were always constant, since the type of symptom expressed was influenced by the effect of the species of *Fusarium*, variety of potato, and by several factors of the environment. These variations are discussed elsewhere in the text.

DEVELOPMENT OF SYMPTOMS IN THE TUBER

Vascular discoloration of the tubers from infected plants was used as the criterion for measuring the effect of the various inoculations and treatments, and while it did not always indicate tuber infection, the association of stem infection and foliage symptoms with tuber discoloration was definite. The amount of the latter arising from chance contamination by fusaria or other causes was estimated from the controls which were placed in one row out of every four throughout the plots. The differences between uninoculated and inoculated lots were ascribed, therefore, to the action of the particular species of pathogen used in the inoculation.

The extent of vascular discoloration in each lot of tubers was recorded in four arbitrary grades as follows: Clean—no vascular browning beyond ¼ inch from the point of stolon attachment; slight—discoloration of all vascular elements up to ½ inch, or of several strands up to 1 inch; moderate—all elements discolored ½ to 1 inch from stolon attachment or a few strands farther than 1 inch; severe—greater degrees of infection than the moderate grade. The percentages of tubers showing symptoms were determined for each lot

and the indices of vascular discoloration were calculated on the basis of the following class weights: 0—clean, 1—slight, 2—moderate, and 5—severe. There was a definite correlation between the percentage of diseased tubers and the tuber-disease index, but the latter was a better measure of severity of infection of the tuber and it correlated more closely with the foliage-symptom index already presented in table 5.

The influence of the method of inoculation on the development of tuber discoloration as expressed by the disease index is shown in table 6. Among the three methods used in 1937 no appreciable difference was noted with *F. oxysporum*, the fresh-cut method was somewhat better in the case of *F. avenaceum*, while the plugged method was definitely inferior to the other two in the case of *F. solani* var. *eumartii*. This is not in accord with the greenhouse inoculation experiments (table 1), where the fresh-cut method gave consistently a higher tuber-disease index for each species than did the presuberized method. In 1939 the fresh-cut method was compared with methods in which inoculated soil and inoculated barley, respectively, were used. The former of these resulted in the highest tuber-disease index with each species, although in the case of *F. avenaceum* and *F. oxysporum* the differences were not great enough to be significant.

TABLE 6.—*The effect of method of inoculation upon development of tuber symptoms as expressed by the tuber-disease index, Madison, Wis., 1937 and 1939*

Method of inoculation	Disease development in tubers from plants inoculated with—						Uninoculated	
	F. avenaceum		*F. oxysporum*		*F. solani* var. *eumartii*			
	1937	1939	1937	1939	1937	1939	1937	1939
	Disease index	*Disease index*	*Disease index*	*Disease index*	*Disease index*	*Disease index*	*Disease index*	*Disease index*
Plugged	6.3	-------	7.2	-------	28.9	-------	0.8	-------
Presuberized	7.8	-------	6.5	-------	44.9	-------	2.1	-------
Fresh-cut	11.5	9.3	6.3	10.3	48.5	25.9	1.3	3.8
Inoculated-soil	-------	12.9	-------	11.8	-------	41.0	-------	5.9
Inoculated-barley	-------	10.6	-------	10.7	-------	31.7	-------	8.5
Average	8.5	10.9	6.7	10.9	40.8	32.9	1.4	6.1

Goss (*8*) reported that early planting increased tuber infection by fusaria in the field. The difference in infection between planting dates in 1937 was measured by the average number of days required for the appearance of foliage symptoms, by the percentage of diseased tubers, and by the tuber-disease index (table 7). Significantly greater infection was produced by planting inoculated seed-pieces on May 25 than on June 9. No difference in the time required for foliage-symptom production appeared between the planting of June 9 and that of June 22, although significant differences between these dates in percentage and severity of the tuber symptoms did appear. It is apparent that the influence exerted by *F. solani* var. *eumartii* is responsible for the differences obtained between the averages of the first two dates of planting. A marked decrease in the amount and degree of infection with all three pathogens was found at the last planting date.

TABLE 7.—*The effect of three planting dates on symptom production by potato plants inoculated with three different species of Fusarium, as expressed by the average number of days required for appearance of symptoms on the foliage, the percentage of diseased tubers, and the disease index of tubers, Madison, Wis., 1937*

[Each figure represents the data from 120 plants]

Date of inoculation and planting	Inoculated with—									Uninoculated			Average		
	F. avenaceum			*F. solani* var. *eumartii*			*F. oxysporum*								
	Days	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*
May 25	119	33.6	11.5	64	73.3	62.5	138	28.7	9.8	196	5.8	1.1	129.3	35.4	21.2
June 9	134	31.6	8.6	92	60.0	36.0	181	29.1	6.5	191	6.2	1.3	149.5	31.7	13.1
June 22	122	19.6	5.6	106	41.7	23.8	156	16.4	3.8	197	6.0	1.8	145.3	20.9	8.7
Average	125.0	28.1	8.5	87.3	58.3	40.7	158.3	24.7	6.7	194.7	6.0	1.4			
Difference required for significance between dates of planting (19:1)													11.7	4.0	2.5
Difference required for significance between species (19:1)													13.5	4.7	2.9

TABLE 8.—*The production of symptoms on four varieties of potato inoculated with three different species of Fusarium, as expressed by the average number of days required for appearance of symptoms on the foliage, the percentage of diseased tubers, and disease index of tubers, Madison, Wis., 1937*

[Each figure represents the data from 90 plants]

Potato variety	Inoculated with—									Uninoculated			Average		
	F. avenaceum			*F. solani* var. *eumartii*			*F. oxysporum*								
	Days	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*	*Days*	*Percent*	*Disease index*
Bliss Triumph	126	11.4	2.8	85	47.1	35.9	171	12.4	3.4	198	4.0	2.0	145.0	18.8	11.0
Irish Cobbler	96	31.1	10.2	64	70.1	53.3	144	24.2	7.1	198	6.1	.8	125.5	32.9	17.9
Katahdin	142	18.9	5.0	92	54.8	36.3	142	18.9	4.5	189	4.1	.9	141.3	24.2	11.7
Rural New Yorker	136	51.1	16.5	108	61.3	37.6	176	43.3	11.7	193	9.8	2.0	153.3	41.4	17.0
Average	125.0	28.1	8.5	87.3	58.3	40.7	158.3	24.7	6.7	194.5	6.0	1.4			
Difference required for significance between varieties (19:1)													13.5	4.7	2.9
Difference required for significance between species (19:1)													13.5	4.7	2.9

The differences in tuber discoloration between varieties of potato are shown in table 8. It is apparent that the expression of foliage symptoms (fig. 7) and the amount and severity of tuber symptoms are inversely related. Early foliage symptoms are found on Irish Cobbler; Bliss Triumph and Katahdin varieties showed symptoms later in the season. The atypical foliage symptoms on Rural New Yorker made accurate recording difficult. Two definite levels of tuber infection were found. Irish Cobbler and Rural New Yorker were more severely affected than were the Bliss Triumph and Katahdin varieties. One early-maturing and one late-maturing variety were included in each group. Previous observations under natural field conditions had indicated that the late-maturing varieties were more subject to disease attack, while the early potatoes appeared to be more resistant or escaped the disease. In the light of these results, the slight difference in resistance between varieties is a function of the individual variety and is not dependent on early or late maturity. The tendency of the early varieties to show very little of the fusarium wilt diseases in Wisconsin is probably due to the fact that they escape the period of most favorable environment for expression of symptoms, which normally occurs late in the season. The differences observed between varieties are in agreement with those reported by Goss (7), who found Bliss Triumph to be more resistant to *F. solani* var. *eumartii* under field conditions than was Irish Cobbler.

Notes were taken previous to maturity on the amount and severity of stem and tuber discoloration in individual plants of Bliss Triumph and Irish Cobbler varieties. Tabulation of these data seemed to indicate a relationship between stem and tuber discoloration. Correlation of these values on a percentage basis is shown in table 9. Positive correlations were found for Irish Cobbler, $r = +0.75$; for Bliss Triumph, $r = +0.49$; and for the total, $r = +0.63$. The values for Irish Cobbler and the total are significant, but the difference between those for Irish Cobbler and Bliss Triumph is not significant. The percentage and severity of stem infection, as the plants infected with *F. avenaceum* and *F. oxysporum* approach maturity, is commonly greater than tuber infection. With *F. solani* var. *eumartii*, percentages and indices of stem and tuber infection more nearly approach the same level. A definite association of foliage symptoms with stem discoloration exists. Individual stems showing aerial symptoms or wilting toward the end of the growing season were usually found to show discoloration of the vascular elements in the below-ground portion of the stem. From the data presented above, one may assume that vascular discoloration of the tubers is an adequate measure of infection where statistically significant differences are obtained following field inoculations.

The data discussed so far with reference to the occurrence of tuber symptoms in field experiments were collected in 1937 and 1939. Those collected in 1938 were not included because a severe epidemic of late-blight tuber-rot in the experimental plot made the analysis of results difficult. A tabulation of the rotting observed in various lots showed the differences in results between varieties of potato but not between the fungus species and controls. For this reason, Rural New Yorker was not included in the analysis, and Irish Cobbler, Bliss Triumph, and Katahdin were treated separately.

TABLE 9.—*Correlation of the percentages of stem and tuber infection on two varieties of potato* [1]

[Each figure represents the data from 30 plants]

Species of pathogen and method of inoculation	Irish Cobbler		Bliss Triumph	
	Vascular discoloration		Vascular discoloration	
	In stems—	In tubers—	In stems—	In tubers—
F. oxysporum:	*Percent*	*Percent*	*Percent*	*Percent*
Fresh-cut	83	22	15	30
Presuberized	52	35	37	17
Plugged	59	43	25	8
F. solani var. *eumartii:*				
Fresh-cut	100	82	87	89
Presuberized	94	80	86	66
Plugged	100	77	79	39
F. avenaceum:				
Fresh-cut	94	59	87	28
Presuberized	83	50	93	18
Plugged	65	27	58	7
Total [2]	730	475	567	302

[1] r_1 for Cobbler = +0.75; r_2 for Triumph = +0.49; r_3 for total = +0.63. At the 5-percent level of significance for: 16° of freedom, r = .47; 7° of freedom, r = .67.
[2] Grand total for vascular discoloration in stems, 1,297; in tubers, 777.

Statistical calculations on the percentage and index of tuber infection showed that *F. solani* var. *eumartii* produced significantly more infection than did the other species, whereas there was no significant difference between avenaceum wilt, oxysporum wilt, and the controls. Since it was possible that the variations caused by late blight rot were masking the differences, a square-root transformation analysis was calculated, in which the square root of the percentage value for each lot was extracted and an analysis was computed from these values (table 10). No attempt will be made to prove the validity of such calculations on these data, but transformation analyses have been successfully used to demonstrate the significance of small differences occurring between samples when these differences were overshadowed by other samples with higher values (*3*). *F. solani* var. *eumartii* again showed significant differences in infection throughout. When transformation values were compared, the percentages of discolored tubers from Katahdin plants inoculated with *F. oxysporum* and from Irish Cobbler plants inoculated with *F. avenaceum* were significantly greater than those from uninoculated plots.

TABLE 10.—*The averages for the percentages and square-root transformation of vascular discoloration produced by the three species of Fusarium on three varieties of potato at Madison, Wis., 1938* [1]

[Each figure represents the data from 120 plants]

Potato variety	Inoculated with—						Uninoculated		Average	
	F. avenaceum		*F. solani* var. *eumartii*		*F. oxysporum*					
	$\sqrt{N}$	*Percent*	$\sqrt{N}$	*Percent*	$\sqrt{N}$	*Percent*	$\sqrt{N}$	*Percent*	$\sqrt{N}$	*Percent*
Bliss Triumph	3.32	14.2	7.84	63.0	4.27	18.5	3.49	12.8	4.73	27.1
Irish Cobbler	3.90	15.3	8.08	65.8	3.52	13.2	1.97	8.0	4.37	25.6
Katahdin	2.68	9.2	4.88	30.8	3.79	13.5	1.50	4.7	3.21	14.5
Average	3.30	12.9	6.93	53.2	3.86	15.1	2.32	8.5		

[1] The differences required for significance between *Fusarium* species and controls at the 5 percent level for percentages in Bliss Triumph is 13.8, Irish Cobbler 11.1, and Katahdin 18.0; for $\sqrt{N}$, 1.0 between *Fusarium* species and controls, 0.9 between varieties and 1.7 (variety×species) for comparing species with each other or with the control within a variety.

RELATION OF ENVIRONMENT TO DISEASE DEVELOPMENT IN THE FIELD

Soil and air temperatures and rainfall were studied in relation to the seasonal development of the three diseases. In 1937, the daily mean air temperature was at or above 24° C. for a period of 38 days, July 11 to August 17 inclusive, and the daily mean soil temperature for the same period was at or above 26°. There was a close correlation between soil and air temperature.

A direct relationship appeared to exist in which temperature, soil moisture, and the amount of infection directly influenced the rate of increase in the severity of symptoms. The effect of environment, while one of predisposition to infection, could not be measured on noninfected plants, and since infection did not occur on all inoculated plants, there seemed to be an element of chance involved in infection and the degree of disease development in the individual plant. The symptoms on one plant, however, ultimately reached the same level of severity as that on any other plant affected by the same disease. It was in this changing picture, rather than in the percentage of infection, that the effect of environment was most apparent.

Low soil moisture, together with high soil and air temperature following infection, increased the severity of disease symptoms and resulted in the rapid collapse of the individual diseased plant. The intervention of a light rain, even though the temperature remained high, extended the period between the first visual symptom and death. These phenomena may be influenced by the rate of transpiration of the plant and the amount of available moisture.

Soil and air temperatures were low in 1938, and periods of rain and cloudy weather were more frequent and more evenly distributed than in 1937. The foliage symptoms of the oxysporum and avenaceum wilts were not distinct. Eumartii wilt, on the other hand, displayed early definite symptoms, and continuous symptom expression occurred throughout the season, although the number of infected plants in 1938 was not so great as in 1937.

Foliage-symptom development at Madison in 1939 was intermediate between that found in the 2 previous years. *F. solani* var. *eumartii* consistently produced symptoms of interveinal chlorosis of the apical leaves, followed closely by leaf-burning and dropping of the lower leaves. Plants affected by *F. avenaceum* and *F. oxysporum* showed only severe leaf-burning in the more advanced stages of the respective diseases. These effects were not generally discernible from drought or leafhopper injury and no definite differences in symptoms could be detected between the two fungus species. The effects, however, were decidedly more pronounced on the inoculated plants than on the control plants. The progression of disease on the individual plant was apparent, as in former years, and resulted in premature death.

In the study of the three inoculation methods, the earliest expression and most rapid increase in severity of foliage symptoms were observed where the barley-kernel inoculum was used. The infected plant was killed in a shorter time after the presence of the disease was indicated. Infection from inoculated soil was not as rapid nor as severe while the dip inoculation produced the mildest type of infection as measured by foliage symptoms.

The temperature at Rhinelander in 1939 was consistently lower than at Madison when the mean daily temperatures of the two locations

are compared (fig. 8). The daily minimum, or night temperature, at Rhinelander was generally 8° to 10° C. lower than at Madison, while the daily maximum, or day temperature, was similar in the two locations. These facts accounted for the similarity in the trends of

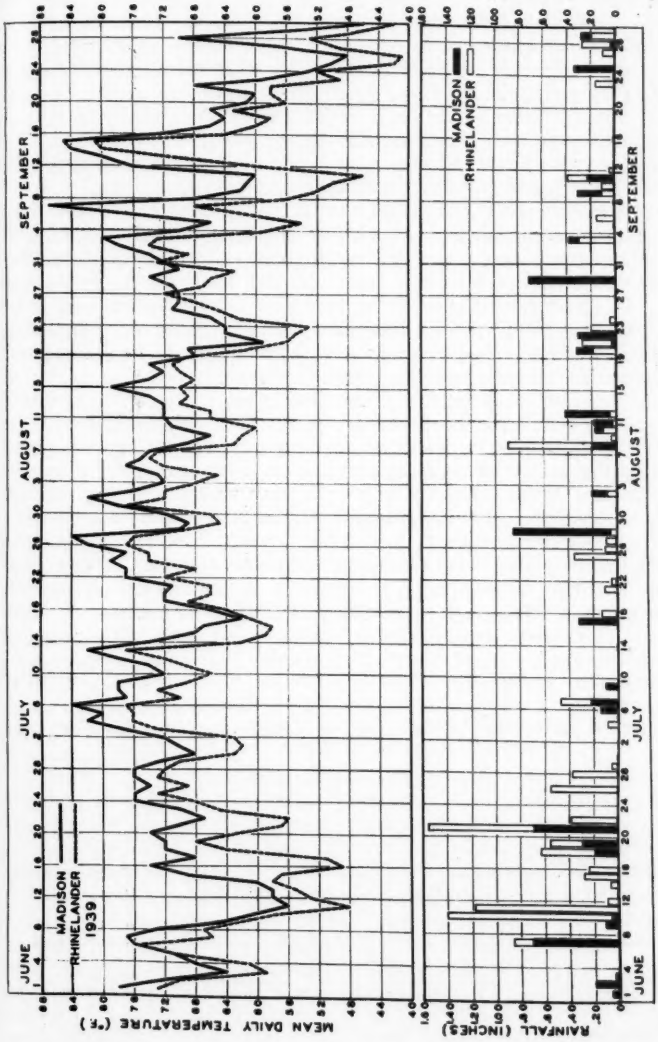

FIGURE 8.—The rainfall and mean daily temperature at Rhinelander and Madison, Wis., for the 1939 growing season.

mean daily temperatures for the two environs, and the lower daily means at Rhinelander. It is possible that the lower mean temperature and the greater daily fluctuation found at Rhinelander were largely responsible for the different symptoms produced in the northern plot.

The infected plants at the northern location showed varying symptoms of yellowing and burning of the leaves throughout July and early August. When notes were taken on August 12, however, there was a marked change in symptom expression. Numerous infected plants of both Bliss Triumph and Rural New Yorker varieties had taken on the characteristic purple-top appearance. There was an upward rolling of the leaf-margins, accompanied by a harsh, almost leathery texture of the leaves. The axillary buds were stimulated into the formation of aerial tubers (figs. 2, 3) which were deep red to purple in color. The adjacent stems and petioles were abnormally thickened and frequently carried traces of the red or purple coloration, as did the apical leaves of infected plants. Aerial tubers were not produced by infected plants at Madison in 1939 nor in previous years.

The 2 weeks preceding August 12 at Rhinelander were characterized by several showers, ending a period of relatively high temperature and low moisture (fig. 8). The average temperature at this time showed a downward trend which was a result of the lower night temperatures. It is possible that the high soil moisture accompanied by cooler temperature is responsible for the production of purple-top symptoms on the plants. Similar symptoms appeared in commercial potato fields of this region at the same time.

The differences between the *Fusarium* species in their symptom production at Starks, in the northern part of Wisconsin, are seen in table 11. Eumartii wilt produced the most severe leaf burning and wilt, while the yellowing and tip burning in plants affected by avenaceum wilt and by oxysporum wilt were greater than in the control plants. A small number of plants infected with *F. solani* var. *eumartii* and *F. oxysporum* produced purple-top symptoms, and a limited number of such plants were found in the control rows. Avenaceum wilt produced the greatest number of plants exhibiting this symptom. All such plants evidenced fusarium infection of roots and stems, from which the respective organism was readily reisolated. In a similar plot at Madison, in the southern part of the State, no symptoms of purple top were produced.

TABLE 11.—*The production of foliage and tuber symptoms on 2 varieties of potato by 3 species of Fusarium at Starks, Wis., 1939*

[Each figure represents 150 plants]

Species of *Fusarium* and variety potato	Plants showing foliage symptoms				Tuber symptoms	
	Wilt or severe leaf burning		Purple-top symptoms			
	Aug. 12	Sept. 3	Aug. 12	Sept. 3		
Bliss Triumph:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Disease index*
F. solani var. *eumartii*	82.5	----------	4.4	----------	54.5	24.7
F. avenaceum	63.0	----------	28.6	----------	23.7	10.5
F. oxysporum	88.0	----------	11.8	----------	22.2	11.6
Uninoculated controls	24.6	----------	3.6	----------	4.5	1.5
Rural New Yorker:						
F. solani var. *eumartii*	79.7	100.0	9.3	7.2	52.1	28.6
F. avenaceum	81.9	85.0	43.2	48.0	29.9	17.2
F. oxysporum	24.0	100.0	5.8	5.8	20.9	8.1
Uninoculated controls	12.0	37.0	3.6	.7	4.9	1.5

The plants showing purple-top symptoms sometimes evidenced a tendency to set underground tubers close to the stalk of the plant. In other such plants, normal tuber setting was found. Tubers from the infected plants displayed vascular discoloration and infection similar to those found in the plot at Madison and with natural infection in the field. The relation between the three fungus species in their ability to cause tuber infection at the Starks plot was similar to that found at Madison in 1939 and in previous years.

DISCUSSION

Examination of the literature discloses many potato disorders which produce similar macrosopic and microscopic effects on the host plant. In a review of leaf roll and associated troubles, Orton in 1914 (*15, p. 19*) stated, "The literature on leaf-roll has become so voluminous that few will undertake to peruse all the contributions, which are, indeed, of very uneven merit, and anyone who attempts it is likely to emerge with his concepts of the disease more confused and hazy than at the start." Since that time the symptoms and modes of transmission of the virus disease known as leaf roll have been defined more fully and the distinction of this disease from others with which it has symptoms very much in common has been more fully recognized. The history of the term "Blattrollkrankheit" has been ably reviewed by Pethybridge (*16*). In recent years, psyllid yellows has been distinguished as a member of the same symptom group, while another, the ring rot disease [*Phytomonas sepedonica* (S. & K.) Bergey et al.] is receiving an increasing amount of attention in this country.

Since a common host reaction is displayed when any one of a number of stimuli is present in a favorable environment, it is not surprising that quite similar symptoms are reported in association with many unrelated causal entities. It is probable that other weak parasites, possibly other species of *Fusarium* not yet described on potato, are capable of causing similar host reactions when conditions of environment favorable for infection are followed by temperatures and soil-moisture conditions which promote the expression of such symptoms. The disease with which *F. avenaceum* is associated is similar in its symptoms to several other diseases but it also differs from them in certain respects.

The foliage symptoms produced by the wilt diseases served as one method of measuring infection. Increase in severity appeared to be a function of the relation between the amount of infection in a plant and climatic conditions which hastened the depletion of soil moisture. A visible association between foliage symptoms and the amount of infection within the stem and roots appeared. A relationship also existed between the amount of stem infection and the amount of vascular discoloration in the tubers.

The time of appearance of foliage symptoms was also used as a measure of disease incidence. The average time which elapsed between inoculation and the expression of symptoms was treated statistically and was found to express the same relationships between various tests as did the data obtained from records on the vascular discoloration of the tubers. Vascular discoloration of tubers as expressed by the tuber-disease index appeared to be a reliable method of expressing the differences between the rates of disease development in the test treatments.

Goss (*5, 6, 8*) has emphasized the fact that *F. solani* var. *eumartii* is a more aggressive parasite on potato than is *F. oxysporum*, and he states clearly the difference between the pathogenicity of these two species. In the field experiments reported in this paper these species together with *F. avenaceum* have received identical treatment in the experiments upon methods of inoculation, dates of planting, and the host varieties tested. Disease records were taken at the same time and in the same manner, and the inoculated plants in any one series were subjected to identical conditions of environment throughout. The individual inoculations were randomized within replicated blocks to minimize any bias and to reduce the error involved in the accumulation of data.

The effect of the pathogenic species on the production of potato wilt and tuber discoloration is seen throughout the tabular presentation of the results of three seasons' work (tables 5 to 11, inclusive). Inoculation with any one species has resulted in a greater amount of vascular discoloration of stems and tubers than was found in the uninoculated plants. The small amount of natural infection in the control plants did not interfere with the statistical treatment of the data nor with the comparison of the three species of wilt pathogens. The relation between the diseases caused by the species of *Fusarium* and the control plants was the same when measured by 50-plant samples in 1939 as by 10-plant samples of the two previous years.

Inoculation with *F. solani* var. *eumartii* resulted in the early appearance of foliage symptoms, a high rate of plant mortality, and a high percentage of severely infected tubers. The percentages and severity of foliage and tuber symptoms were consistently greater, and were frequently double that exhibited by plants inoculated with *F. oxysporum* or *F. avenaceum*. Eumartii wilt was influenced most by different dates of planting, by different methods of inoculation, and by the varieties of the host plant used, although similar trends were recorded for avenaceum wilt and oxysporum wilt. *F. solani* var. *eumartii* is capable of attacking the host plant under conditions of moisture and temperature which are optimum for the development of the host.

F. oxysporum was a less aggressive parasite than *F. solani* var. *eumartii* and the effects of oxysporum wilt were apparent later in the growing season. In this respect and in other ways, *F. avenaceum* and *F. oxysporum* were similar. Both avenaceum wilt and oxysporum wilt are late-season diseases in Wisconsin, and both produce the greatest wilting effect on the host plants either during or following a period of hot, dry weather. *F. avenaceum* and *F. oxysporum* were similar in infectivity when the diseases were measured by foliage symptoms or by the percentage and severity of discoloration in the tubers. No significant statistical differences were found between the disease effects produced by these two fungi. Infection in the control plants was consistently lower and infection by *F. solani* var. *eumartii* was always higher than that produced by either avenaceum wilt or oxysporum wilt.

The similarity of behavior of *F. avenaceum* and *F. oxysporum* observed throughout this series of experiments is of interest when the growth-temperature relationships of the two fungi are considered. A difference of 6° to 8° C. was found for the optimum growth of the

two species on artificial media. However, the conditions of temperature and moisture which favored infection and disease development were similar for the two pathogens.

Differences found between inoculation methods could generally be correlated with the abundance of fungus inoculum near the roots of the potato plant. The effect of allowing the seed pieces to heal before inoculation was not as great as was to be expected and did not influence the percentage of diseased tubers. The severity of disease in the tubers was greatest and foliage symptoms appeared earliest when seed pieces were inoculated by dipping in a spore suspension or when inoculated soil or inoculated barley kernels were placed in proximity to the seed piece at the time of planting.

Early death of the plant due to seed-piece infection by wilt fusaria was not found commonly under Wisconsin conditions. This effect was observed only when a large amount of inoculum was placed in contact with the seed piece. Favorable soil moisture and temperature at planting time probably aid in the formation of wound cork and allow the plant to become established to the extent that seed-piece infection does not result in early death.

The effect of different planting dates on the incidence of the wilt diseases may be partially accounted for by the favorable influence of the moist soil early in the season. Cool soil temperatures were especially favorable to infection by *F. solani* var. *eumartii* at the early planting date, while soil moisture had decreased and soil temperatures had increased at the later dates of planting.

The differences between potato varieties in the amount of wilt observed is in agreement with previous reports in the literature. These differences may vary, however, with the environment, since changes in date of planting and harvest, recording the disease data, and other factors may influence differently the level of infection determined for each variety.

Leaf rolling, reddening and rosetting of the foliage, and production of axillary aerial tubers are common with avenaceum wilt in northern Wisconsin. These effects are not usually observed in the southern part of the State. Cool night temperatures or a fluctuating daily temperature appear to play some part in the production of these purple-top symptoms when the soil moisture is sufficient to prevent rapid desiccation and wilting of the infected plant.

SUMMARY

Numerous diseases of potato have been described, the symptoms of which exhibit striking similarity to the various stages of the wilt diseases caused by *Fusarium avenaceum*. The symptoms of avenaceum wilt are compared with those of other diseases, including eumartii wilt and oxysporum wilt. *F. avenaceum* is compared with both *F. oxysporum* and *F. solani* var. *eumartii* throughout the paper.

A strain of *F. avenaceum* isolated from diseased potato plants was capable of infecting and producing vascular discoloration of the roots, stems, and tubers of inoculated plants under both greenhouse and field conditions. Several single-spore lines of this culture differed slightly from each other in their attack on the host plant.

Penetration of the roots occurred rapidly when inoculated with each of the three pathogenic species. Hyphae of each of the fungi

were found in the roots and lower stem infected plants. The mycelia of *F. oxysporum* were closely confined to the xylem vessels of the stem; those of *F. avenaceum* occurred abundantly in both the vascular and cortical tissues of the lower stem, while the mycelia of *F. solani* var. *eumartii* were most abundant in the stem cortex. Heavy-walled xylem cells filled with a dense granular deposit and disintegration of certain cells of the phloem and xylem of the stem were found to be associated with infection by each of the three pathogens. Abnormal effects in the host tissues in advance of fungal invasion were greatest in plants inoculated with *F. solani* var. *eumartii*.

The air temperatures which favored optimum growth of potato and of the strain of *F. avenaceum* used were found to be between 20° and 24° C. A higher soil temperature (28°) was most favorable for infection of the potato plant. A moist condition of the soil, with the water-holding capacity maintained at 50 per cent, was more conducive to infection than were either dry or wet soils with the moisture capacity at 30 or 70 percent, respectively.

Inoculation of the seed pieces at planting time was used successfully to induce development of the three wilt diseases in the field experiments. The effects of various treatments were measured by the time required for foliage symptoms to appear and the severity of tuber symptoms. These two criteria of infection were treated statistically. Differences occurring between methods of inoculation appeared to be closely correlated with the amount of inoculum in proximity to the plant.

Irish Cobbler and Rural New Yorker varieties were more susceptible to attack by the wilt fungi than either Bliss Triumph or Katahdin.

Early planting of inoculated seed pieces resulted in a greater percentage of infection and in more severely diseased tubers than did late planting.

Eumartii wilt was the most aggressive and resulted in the greatest percentage of severely infected plants. *F. avenaceum* was similar to *F. oxysporum* in the percentages of infection, the severity of disease, the time of appearance of foliage symptoms, and the temperature most favorable for infection. The two pathogens differed slightly from each other in their temperature requirements for growth and the symptoms produced on infected plants.

Infected plants at Starks, in the northern part of the State, displayed leaf rolling, reddening and rosetting of the foliage, and aerial tubers in the axils of the leaves. This type of symptom was not pronounced in a similar plot at Madison in southern Wisconsin. The greatest percentage of plants showing purple top resulted from inoculation with *F. avenaceum*, some were found associated with oxysporum wilt, but few with eumartii wilt.

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MICROBIAL RESPONSES TO ORGANIC AMENDMENTS IN HOUSTON BLACK CLAY [1]

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INTRODUCTION

King (*5*)[3] and various colleagues in a series of papers reported the control of cotton root rot (*Phymatotrichum omnivorum* (Shear) Dug.) by the application of large amounts of stable manure to irrigated plots of alkaline soil in Arizona. Preliminary results, especially those obtained by the use of Cholodony slides (*6*), pointed to great increases in the microflora of the soil as accompanying, perhaps causing, the reduction in growth and activity of the root rot fungus. Since the same disease has produced losses in the cotton crop running into millions of dollars each year for over half a century in Texas (*3*), study of soil microbiological aspects of the problem under Texas conditions was necessary.

The soils infested with the root rot organism in Texas belong to the Houston series and related soil types. These soils constitute the black land prairie areas of Texas; they are alkaline in reaction, ranging from about pH 8 to 8.5 or even 8.7. They form a group of highly colloidal and close-textured calcareous types, with high moisture-holding capacity and with a tendency to become very hard and to crack in dry periods. Thus they present a pronounced contrast to the northern and eastern soils, which have formed the basis for most studies of the microbial floras of American soils.

Microbiological studies of Houston soils, exclusive of those dealing with the distribution of the *Phymatotrichum* fungus, have been limited. Werkenthin (*14*) isolated and identified some of the saprophytic molds of the Austin region; Morrow et al. (*8*) isolated and identified the mold flora of another area; Williams (*15*) noted some bacterial contents of virgin and cultivated soils; and Lewis (*7*) determined the distribution of green fluorescent bacteria in several Texas soils, including Houston clay. As a background for planning further experimentation with these difficult soils, a comparative study of microbial floras under environmental conditions presented by certain selected plots in cotton fields is reported here.

EXPERIMENTAL PROCEDURE

Field-soil samples were taken periodically from November 11, 1937, to December 15, 1938, from plots under different types of crop man-

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[2] In this work the senior author acknowledges the assistance and counsel of H. V. Jordan, of the Bureau of Plant Industry, and Professors I. M. Lewis, G. W. Goldsmith, and Marie B. Morrow, of the University of Texas.

[3] Italic numbers in parentheses refer to Literature Cited, p. 533.

agement. Two of the three plots chosen for study were from an experiment designed for comparing the effect of turning under a second crop of sorghum with the effect of continuous cotton cultivation upon the incidence of root rot of cotton. These plots were located in Soil Fertility's [4] experiment No. 12 on Blank's plantation, near Lockhart, Tex. Sorghum residues, turned under on October 25, 1937, were estimated at about 3,000 pounds, dry weight, per acre. A third plot was chosen from another Soil Fertility experiment on the Voelker farm near Kimbro, Tex., on which cowpeas were grown in 1937 and the entire crop turned under on October 18, 1937. The area carrying cowpeas had not been selected for this experiment at the time the crop was turned, so the amount of material turned is not known. The growth was good. All three plots were cropped to cotton in 1938.

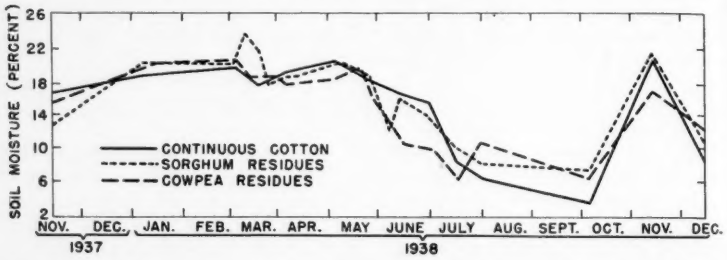

FIGURE 1.—Soil moisture, as determined by air-drying, for the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

Soil samples were collected from approximately 4-inch and 12-inch levels; each soil sample was a thoroughly mixed composite of roughly equivalent soil portions taken from four separate locations. Soil and air temperatures were taken at the time of collection of field samples. Cultures were made from moist soils as soon after collection as practicable. The total number of micro-organisms was determined from Petri-dish cultures on sodium albuminate agar; the number of actinomycetes on glycerol nitrate-soil extract agar; and the number of filamentous fungi on acid-dextrose-peptone agar. Spores of aerobic bacilli were determined by plating out the appropriate dilutions on nutrient beef agar after pasteurization at 80° C. for 10 minutes. Quantitative estimates of blue-green fluorescent bacteria were made by the dilution-tube method, on an asparagine medium, and the most probable numbers were determined from the tables of Halvorson and Ziegler (*4*). Soil moisture (fig. 1) was determined by air-drying 100-gm. portions of moist soil; the observed loss in weight was expressed as percentage of moisture in moist soil.

RESULTS

Population changes for various microbial groups found in Houston black clay at different seasonal dates and under different conditions of soil management are presented graphically in figures 2 to 6 for the

[4] Former Division of Soil Fertility Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

4-inch level of sampling. The results obtained from samples taken at the 12-inch level followed the same general course. The numbers of micro-organisms were conspicuously lower than those for the upper level. Although the figures were obtained and tabulated they are omitted here as not changing the significance of the studies made at the 4-inch level.

TOTAL PLATE COUNTS

The period of maximal activity for all plots, as indicated by the total counts (fig. 2), was found to begin in March and to end early in June. The greatest activity was shown in soil receiving organic residues. The highest total counts in the plots receiving sorghum residues were 428.4 million per gram of dry soil, and for plots receiving cowpeas, 430.7 million, whereas the largest population observed in the continuous-cotton soil was 179.4 million.

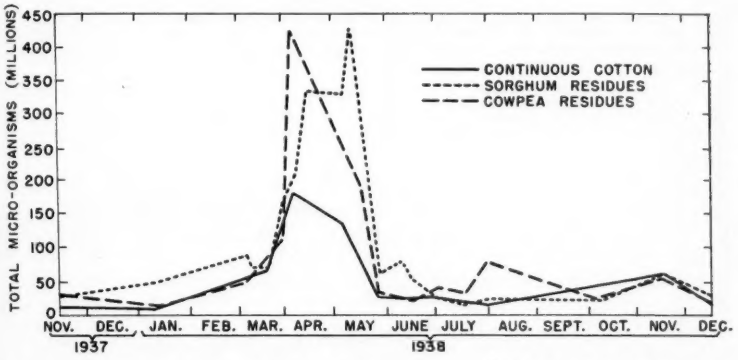

FIGURE 2.—Total counts of micro-organisms in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

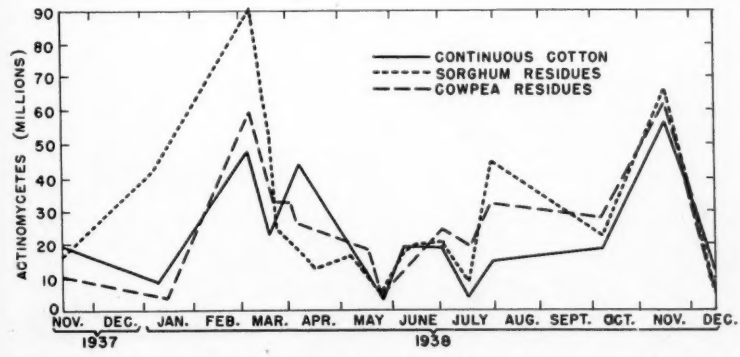

FIGURE 3.—Counts of actinomycetes in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

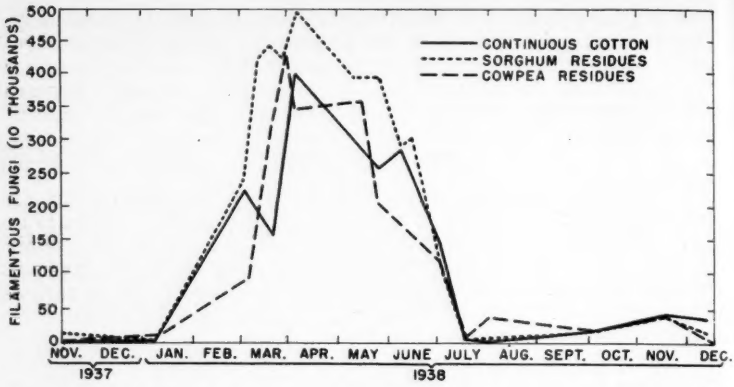

FIGURE 4.—Counts of filamentous fungi in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

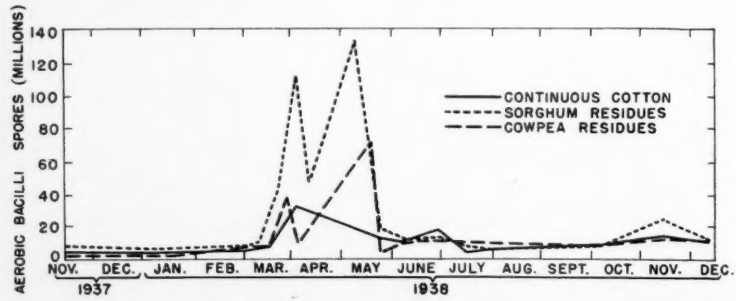

FIGURE 5.—Counts of aerobic bacillus spores in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

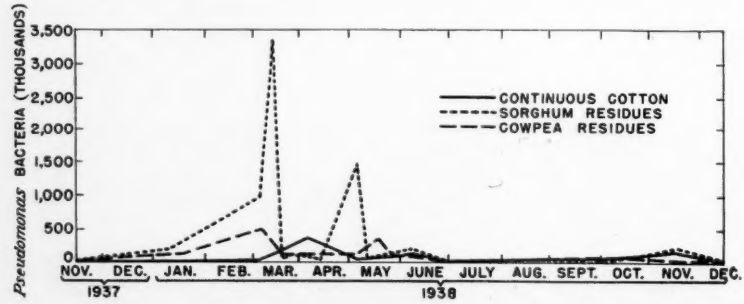

FIGURE 6.—Counts of *Pseudomonas* bacteria in the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

The numbers of micro-organisms found in these samples of Houston black clay are greater than those generally reported in studies of soil. Waksman (*13*) has reported from 7 to 10 million total bacteria per gram for rich garden and cultivated orchard soils in New Jersey; Erdman's data (*2*) on Iowa Carrington loam reveal generally 7 to 15 millions per gram; and Vandecaveye (*12*) reports from 3 to 20 millions per gram in his studies on Palouse silt loams in Washington.

FUNGI

The fungi, like the bacteria, showed extensive increases during the spring months. This increase in fungus population became apparent earlier in the season and continued later into the summer than did that of other microbial populations (figs. 2 and 4). The number of fungi in the three soil treatments studied showed smaller differences according to type of soil management than did those of other groups of micro-organisms considered.

ACTINOMYCETES

Increased numbers of actinomycetes appeared during the late winter period in all plots (fig. 3). In early March, the colony count per gram of soil reached 47.5 million for the continuous-cotton soil, 89.5 million where sorghum residues were added, and 59.7 million where the cowpeas were plowed under. The fluctuations shown in colony count of actinomycetes have not been explained, but the totals involved are strikingly greater than those shown in previously reported studies (*2*, *13*).

In a subsequent experiment at the Greenville station, sorghum buried deeply in bundles over which cotton was planted was dug out in the last week of August 1939. As the area was broken open the odor of *Actinomyces* was detected strongly, and sorghum stalks only partly decomposed were powdery white with *Actinomyces* spores. In the buried bundles, the presence of open spaces or cracks was an obvious factor in the development of powdery spore masses. Temperatures in the soil, 10° to 20° higher than those found in northern and eastern soils, may be an important factor in the totals found.

SPORES OF BACILLI

Changes in the number of spores of aerobic bacilli (fig. 5), under different seasonal conditions and under different types of soil management, parallel roughly the changes observed for total microbial populations. Maxima were obtained during the spring period; thus the effect of organic residues was apparent. The maxima for unamended soil and for treatments with sorghum and cowpea residues were 31.4 millions, 132.4 millions, and 72.5 millions, respectively.

The blue-green fluorescent bacteria (fig. 6) showed response to added organic residues during the latter part of the winter. Maxima observed for the sorghum- and the cowpea-residue amended soil were 3,400 thousands and 500 thousands, respectively, and for the unamended soil, 370 thousands. The first two values were observed in early March, and the last in early April. Like total bacteria and sporulating bacteria, *Pseudomonas* types showed depressed numbers during the summer and early fall months.

DISCUSSION AND INTERPRETATION OF RESULTS

Study of figure 7, showing the general temperature relations through 1 calendar year, shows that soil temperatures at the 4-inch level fell below 60° F. about December 1, 1937, and again reached 60° early in February, and 70° by March 1, 1938. At no time were freezing temperatures observed. Nevertheless, the cooler temperature shown would readily account for the fact that the total number of microorganisms was static (fig. 2) from the latter part of November to the

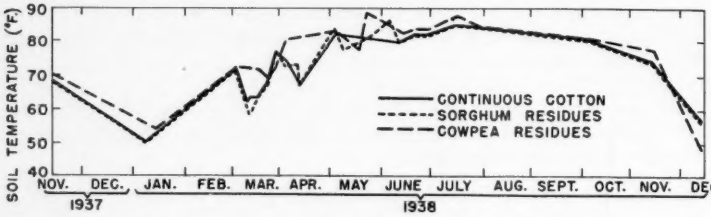

FIGURE 7.—Soil-temperature readings for the 4-inch levels of soil receiving continuous cotton cultivation, and sorghum- and cowpea-residue amendments.

early part of March. Very low moisture content in the latter part of July to October clearly correlates with low bacterial activity during that period. Such observations are in entire harmony with the observations of many workers (*1*, *2*, *4*, *9*, *13*).

Comparison of figures 1, 2, and 7 shows a rather sharp fall of temperatures beginning about November 1, 1937, accompanied by small changes in bacterial population, as indicated by the colony count in Petri-dish cultures. Although there was a differential rise in number of actinomycetes during the winter months (fig. 3), the total flora as shown by colony count (fig. 2) changed little before the temperature rose to 70° F. again early in March. The abrupt maxima reached in April and May can therefore be interpreted as the resumption of bacterial decomposition in the organic matter plowed under late in October. This activity would appear to have been arrested by the falling temperatures. The peak number for the 4 spring months, reaching more than 400 million micro-organisms, represents the normal rise where such amounts of plant materials are plowed under (compare Smith and Humfeld, *10*, *11*). It is noteworthy that in the plot receiving the residues from the previous cotton crop alone, the rise was simultaneous, even though the highest figure was somewhat under half (179 millions) of that for the green crops plowed under.

Transferred to Texas conditions, King's hypothesis that the introduction of masses of organic matter would produce great microbial activity is fully confirmed. The numbers reported here from colony counts in Petri-dish cultures are strikingly higher even than those of Smith and Humfeld (*10*, *11*) from their composite samples.

SUMMARY

Curves for temperature, moisture, and microbial numbers were established for selected plots of Houston soil as a background for

studies in root rot control. As an environment for microbial activity, the plots of Houston soil studied furnish the following striking features:

(1) In a period between early December and February the temperatures remained below those required for active microbial multiplication, yet without freezing.

(2) Temperatures ranged above 70° F. from March to November, and from 80° to 90° from June to October.

(3) Bacteria and actinomycetes are much more active and abundant than has been reported for northern soils. Maxima for total colony counts in soils receiving organic amendments reach 200 to 400 millions; actinomycetes at times reach 50 and 90 millions to the gram. Such responses to added organic nutrients present a challenge to the worker to search for organic media and agronomic practices capable of yielding a controlled microflora.

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SOIL BACTERIOLOGICAL STUDIES ON THE CONTROL OF THE PHYMATOTRICHUM ROOT ROT OF COTTON [1]

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INTRODUCTION

Search for methods of control or elimination of the cotton root rot fungus (*Phymatotrichum omnivorum* (Shear) Dug.) in the alkaline soils of the Southwest has already continued for half a century. The literature involved has recently been reviewed by Rea (*16*)[3] and by Streets (*17*). Nearly all of these discussions have been from the viewpoint of plant pathology or agronomy. Garrett (*7*) included this disease in his broad discussions of root-disease problems. Observations pointing to a soil microbiological factor began with Pammel's (*14*) observation that the incidence of this disease was lessened by liberal use of stable manure. Actual predication of soil microorganisms as a factor in the conquest of the disease was offered by King and his colleagues (*8, 9*) in a series of papers upon the manure treatment of root rot plots in Arizona, beginning in 1923 and definitely proposing the microbiological interpretation of such control in 1934. Search for antagonistic organisms effective in the cotton field has thus far proved futile (Morrow et al., *12* and unpublished data), although such organisms are readily demonstrated in pure culture. Up to the present, no antibiotic agent has been able to destroy or render static a root rot fungus already established as a parasite in the cotton plant. The work discussed in this paper, therefore, deals with the relations of the soil micropopulation to the root rot fungus in the soil and in dead or decaying roots, not in its active phase as a parasite.

The persistence of *Phymatotrichum omnivorum* on cotton roots under different soil or root conditions and the interpretation of the observed differences have remained subjects for speculation. King and Loomis (*10*), McNamara and Hooton (*11*), and Ratliffe (*15*) have observed that the parasite is viable for considerable periods after its invasion of cotton roots. That its viability is decreased under certain conditions is also known. Rea (*16*) has stated that the older fungus strands on diseased and dying portions of cotton roots gradually exhaust the available food materials therein and die with the roots to which they are attached. Ezekiel (*5*) has noted decreased survival percentages of *P. omnivorum* on cotton roots following root cutting or girdling, as well as a decrease in alcohol-soluble solids contained in the roots. Such studies suggest that food exhaustion or changes in root composition become limiting factors for survival of the parasite.

The role of microbial interrelationships in determining the survival of plant pathogens has been reviewed by Garrard and Lochhead (*6*). King, Hope, and Eaton (*9*) have discussed the importance of microbial factors in the manurial control of cotton root rot on irrigated land. Clark and Thom (*4*) concluded that the suggested microbiological

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[2] The authors express their deep appreciation to Charles Thom, principal mycologist in charge, Division of Soil Microbiology for his many valuable suggestions during the course of these studies.
[3] Italic numbers in parentheses refer to Literature Cited, p. 547.

sanitation effects of manurial amendments must be accomplished through the agency of the soil microflora; the root microfloras themselves showed no appreciable changes following organic amendments. Recently Adams, Wilson, Hessler, and Ergle (*1*) reported that early fall plowing of cotton stalks checked the destructiveness of the fungus in the following cotton crop. The extent to which root microfloras influence the survival of *Phymatotrichum omnivorum*, and in turn the extent to which microfloras associated with cotton-root surfaces are influenced by plant injuries inflicted by early plowing or girdling, remain unreported.

It is known that sclerotia of the cotton root rot fungus may remain in field soil as viable and infectious material for many years, even when fields are maintained in nonsusceptible crops or in clean fallow. Sclerotia have been considered important in the survival of primary infection centers; their elimination, therefore, becomes desirable in soil sanitation.

Certain physical conditions, such as soil desiccation or aeration, have been reported to influence the survival of sclerotia (*8, 13, 18*). The successful manurial control of several soil-borne diseases (*7*) and the possibility that microbial relationships contribute to the elimination from soil of plant pathogens raise the question of the ability of sclerotia of *Phymatotrichum omnivorum* to survive when exposed to the microbial activity associated with fresh organic residues in soil.

It has been the purpose of the following investigations to consider (1) the growth and persistence of *Phymatotrichum omnivorum* on cotton roots or root segments under different environmental conditions and (2) the elimination of *P. omnivorum* sclerotia from soil, with especial reference to the periods of microbial activity following organic fertilizations.

EXPERIMENTAL PROCEDURE AND OBSERVATIONS

GROWTH AND PERSISTENCE OF PHYMATOTRICHUM MYCELIUM ON COTTON-ROOT SEGMENTS IN AMENDED LABORATORY SOILS

Indications of the importance of the effects of environmental factors on *Phymatotrichum omnivorum* were readily seen when freshly infected roots were collected from field cotton and placed in contact with differently treated soil in the laboratory. A representative experiment is summarized briefly.

Ninety-six quart jars, in 4 batteries of 24 jars each, were half-filled with Hunt clay. The first battery was retained unamended; the second, third, and fourth batteries received 1 percent superphosphate, 3 percent barnyard manure, and 3 percent chopped sorghum fodder, respectively. Four jars in each battery were brought to optimum moisture content immediately and incubated at a controlled room temperature of 25° C. for 30 days. Additional sets of 4 jars in each battery were moistened at intervals, so that, after 30 days had elapsed, jars were available to represent periods of incubation of 0, 5, 10, 15, 20, and 30 days for each of the 4 soil conditions. All soil containers were then inoculated with *Phymatotrichum omnivorum;* 2 jars in each block of 4 received agar-culture inoculum, and the other 2 replicates in the block received infected root segments. Observations on the growth

of *P. omnivorum* mycelium and on its persistence after development were made at 3, 5, 10, and 16 days after inoculation.

Mycelium of *Phymatotrichum omnivorum* developed quickly over the soil surfaces in jars containing unamended and superphosphate-amended Hunt clay. The mycelium produced showed little disintegration during an observation period of 16 days, and random subcultures to new check soils consistently showed viability of the fungus. In contrast, infected roots or agar inocula placed on soil containing organic materials either failed to grow or showed initial growth followed by disintegration of the mycelium. The soil lots amended with manure and with sorghum fodder differed somewhat both in their periods of mycelial inhibition and in their associated soil micropopulations. The presence of manure in the soil inhibited growth or persistence of *P. omnivorum* mycelium from inocula only within the first 15 days after its application; after incubation for 20 days or longer, manured soil permitted growth of mycelium. Sorghum fodder in soil prevented growth of the fungus for the full 30-day period following its application. For both amendments, the period unfavorable to mycelial growth showed close agreement with the period of intensified microbial activity. Greater and more prolonged differences were noted for the soil micropopulations following fertilization with sorghum residues; after 30 days the soil micropopulations in such soils were still approximately seven times those of check soil. For manured soil, micropopulations were slightly more than double those of the check soil for the first 2 weeks after treatment but showed little difference thereafter.

Other experiments, established under conditions similar to the preceding series but with different types of amendments, showed that decomposing organic materials generally, if capable of supporting increased microbial activity, induced situations in which mycelial growth from freshly infected cotton roots was inhibited completely or else destroyed rapidly after an initial development. Among other organic materials, cottonseed meal and hulls and chopped cotton roots were found to be suitable treatments for inhibiting mycelium.

On Cotton Roots in Field Experiments

The response of the root-surface microflora to various types of root injury was studied in field experiments. In order to establish a background of information concerning normal root-surface micropopulations, before attempting to define those of injured roots, a seasonal study of the microfloras associated with cotton of different ages and of different dates of planting was started May 3, 1940, and was continued for 6 months. Field samples consisted of composites from a minimum of five plants; the root systems collected by excavations were separated into crown, deeper tap, and lateral portions for laboratory studies. Microbiological analyses were made according to the methods previously reported by Clark (*2, 3*), except that whole root segments rather than root-surface scrapings were employed in the primary dilutions. Surface areas were determined as accurately as possible by measurements, and the microbial populations were expressed as number of micro-organisms per square centimeter of root surface. The seasonal microfloras associated with surfaces of uninjured cotton roots are shown in table 1.

TABLE 1.—*Microfloras associated with the surfaces of healthy cotton roots, season of 1940*

Date of planting cotton	Date of root sampling	Age of cotton sampled	Microbial groups determined															
			Total microbial number [1] on—			Dye-tolerant bacteria on—			Filamentous fungi on—			Spores of bacillus on—			Fluorescent bacteria on—		
			Crown roots	Tap-roots	Lateral roots	Crown roots	Tap-roots	Lateral roots	Crown roots	Tap-roots	Lateral roots	Crown roots	Tap-roots	Lateral roots	Crown roots	Tap-roots	Lateral roots
		Days	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Thousands*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*	*Hundreds*
Apr. 16	May 3	17	[1] 2,850			2,500			4.8			104.0			7,960.0		
	May 15	29	1,650			450			51.0			8.3			510.0		
	June 22	67	335	94	58	39	20		13.4	2.2	4.0	3.1	4.7	5.2	2.9	6.3	9.2
	July 5	80	522	127	22	194	16	4	2.7	.9	1.3	8.9	10.2	8.3	26.6	4.9	.1
	July 11	86	464	117	39	740	56	47	15.3	.8	.9	1.7	4.2	5.2	48.1	.1	1.3
	July 26	101	131	15	10	175	10	10	4.5	2.3	.3	3.3	1.9	3.7	16.1	.1	.1
May 7	July 11	65	608	150	138	382	263	36	1.9	2.0	1.7	2.7	4.7	2.0	48.4	.7	2.3
	July 18	72	414	91	101	197	42	71	1.1	.6	.4	9.6	4.3	5.2	74.4	.8	.9
	July 25	79	410	191	156	164	61	18	2.0	.7	.6	3.4	5.7	3.1	42.8	2.5	.2
	Aug. 15	100	34	124	11	63	147	14	3.2	.1	.3	4.7	4.0	2.4	17.6	1.6	1.2
June 4	June 22	18	333			37			13.0			8.5					
	July 5	31	1,259			355			2.7			5.9			30.9		
	July 11	37	1,280	283	160	1,375	358	100	330.0	2.4	2.7	4.4	6.0	1.5	920.0	20.8	37.6
	July 18	44	438	57	106	239	28	52	1.6	1.8	1.3	4.6	2.1	3.6	20.4	1.6	9.0
	July 25	51	577	54	243	193	11	35	2.0	.3	2.3	6.9	4.7	7.8	94.8	.5	3.0
	Aug. 15	72	60	17	19	48	14	30	1.9	.2	.4	1.6	1.1	1.3	9.0	.1	.2
	Sept. 20	108	278	91	275	181	35	222	3.1	1.4	2.8	8.7	4.4	7.3	4.7	.1	42.3
	Sept. 27	115	265	64	143	220	50	103	3.2	.8	.5	10.9	4.1	1.6	2.7	.4	1.7
	Oct. 18	136	293	109	128	230	72	82	3.5	1.7	2.0	7.4	5.3	2.3	41.5	1.9	2.2

[1] Number of micro-organisms per square centimeter of root surface.

Young cotton plants possessed somewhat higher root micro-populations than did older plants; the latter, when maintained uninjured under the usual practices of continuous cotton cultivation, had root microfloras that did not differ materially during the late fall from those observed during the early fall or late summer. In contrast, greatly intensified microbial activity was observed for root surfaces of injured cotton plants. Representative data for different types of injury are presented in table 2. Injuries inflicted during the late fall had less effect on root-surface microfloras than did late-summer injuries. Relative increases of total microbial numbers following injuries to roots on August 19 over those for uninjured root systems are shown in table 3. Differences in the gross appearance of such roots were easily apparent (fig. 1).

TABLE 2.—*Microfloras associated with the taproots of uninjured and injured cotton plants, season of 1940*

Date of sampling	Microbial groups determined	Micro-organisms per square centimeter of root surface of plants—							
		Not injured	Cut 4 inches below crown			Cut 4 inches above crown			Girdled 4 inches above crown, Aug. 19
			Aug. 19	Sept. 19	Oct. 19	Aug. 19	Sept. 19	Oct. 19	
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Aug. 19	Total microbial number.	16,550							
	Dye-tolerant bacteria	14,400							
	Filamentous fungi	22							
	Spores of bacillus	105							
	Fluorescent bacteria	14							
Sept. 20	Total microbial number	90,500	14,500,000			350,000			8,800,000
	Dye-tolerant bacteria	35,000	14,200,000			112,000			6,000,000
	Filamentous fungi	140	825			230			68
	Spores of bacillus	441	1,039			737			538
	Fluorescent bacteria	3	72,900			25			66
Oct. 18	Total microbial number	109,000	17,200,000	80,000		55,000	72,000		231,000
	Dye-tolerant bacteria	72,000	16,400,000	26,000		55,000	47,000		156,000
	Filamentous fungi	174	1,098	1,679		65	97		1,390
	Spores of bacillus	526	448	644		227	767		1,307
	Fluorescent bacteria	193	462,400	65		28	154		1,173
Nov. 19	Total microbial number	28,000	7,100,000	319,000	53,000	95,000	92,300	32,000	385,000
	Dye-tolerant bacteria	39,300	4,700,000	345,000	53,000	7,300	80,300	43,700	158,000
	Filamentous fungi	560	4,700	600	100	90	46	60	330
	Spores of bacillus	870	4,800	2,100	1,100	520	850	440	2,200
	Fluorescent bacteria	1,800	128,400	81,300	78	200	29,800	800	300

TABLE 3.—*Comparative total microbial counts on surfaces of uninjured and injured cotton roots at 1, 2, and 3 months after injury on Aug. 19, 1940*[1]

Date of sampling	Check (uninjured)	Cut below the crown	Girdled above the crown
Sept. 20	1	160	97
Oct. 18	1	158	2.2
Nov. 19	1	254	14

[1] Micropopulations on surfaces of injured roots compared with those on healthy uninjured roots (taken as unity) on corresponding dates of sampling.

On Cotton Roots in Different Stages of Decomposition

In a concluding experiment, the availability of cotton roots at different stages of decomposition as nutrient material for *Phymatotrichum omnivorum* was determined. Cotton roots or root segments that had undergone periods of decomposition from 0 to 350 days were collected from 29 different laboratory and field experiments. Each collection of root material was divided into 3 equal parts; these were moistened and placed in Erlenmeyer flasks. The first flask of each set of 3 was left unsterilized; the 2 remaining flasks were autoclaved until sterile. One of these was maintained sterile thereafter, except for the addition of *P. omnivorum;* the other was inoculated with unsterilized soil. Standard agar disks of *P. omnivorum* were introduced into all flasks, and observations for growth of mycelium were made during the following 2 months. The source and treatment of the cotton roots employed are shown in table 4.

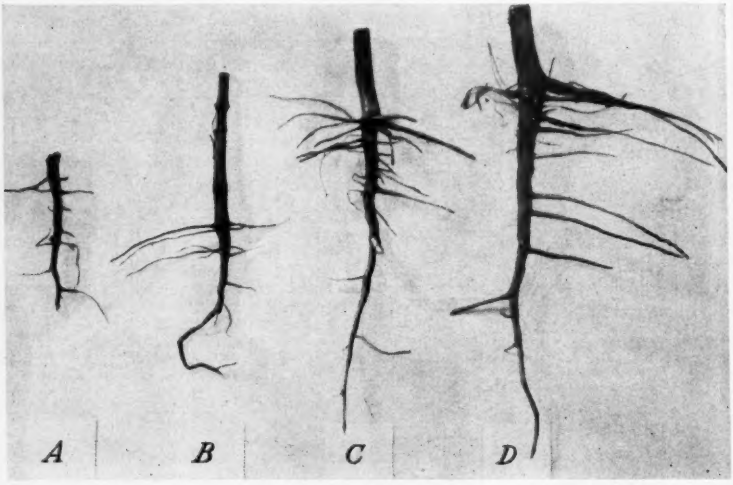

Figure 1.—Appearance of cotton roots injured on August 19, 1940, and excavated November 18, 1940: *A*, Clipped below the crown; *B*, girdled above the crown; *C*, clipped above the crown; *D*, uninjured cotton root (check) from the same experiment.

Without exception, in the unsterilized flasks *Phymatotrichum omnivorum* mycelium failed to develop upon any root material, regardless of the extent of decomposition previously undergone by the root segments. Mycelium developed in all flasks sterilized and then subsequently inoculated with *P. omnivorum* only; in such flasks the competitive effect of other micro-organisms was absent. Mycelium failed to develop in those flasks sterilized and then reinoculated with a pinch of nonsterile soil together with *P. omnivorum;* such results showed that the growth obtained in the pure-culture series did not occur simply because of the liberation of additional food materials during the sterilization process.

TABLE 4.—*Types of cotton roots studied for their ability to support growth of Phymatotrichum omnivorum*

Condition of root material and treatment number	Treatment undergone by the cotton roots	Period of decomposition before experiment was established
		Days
Healthy:		
1	None	0
2	Buried in unamended soil	7
3	do	14
4	do	30
5	do	45
6	do	60
7	do	75
8	do	90
9	do	350
10	Buried in manured soil	350
11	Buried in sorghum-fodder-amended soil	350
12	Buried in 15-0-0-amended soil	350
13	Buried in 0-15-0-amended soil	350
14	Injured by cutting above crown and left in field soil	11
15	do	40
16	do	70
17	Injured by cutting below crown and left in field soil	11
18	do	40
19	do	70
20	Injured by girdling above crown and left in soil	70
Diseased:		
21	Buried in unamended soil	350
22	Buried in manured soil	350
23	Buried in sorghum-fodder-amended soil	350
24	Buried in 15-0-0-amended soil	350
25	Buried in 0-15-0-amended soil	350
26	Buried in unamended soil	273
27	Buried in manured soil	273
28	Buried in sorghum-fodder-amended soil	273
29	Buried in 0-15-0-amended soil	273

ELIMINATION OF PHYMATOTRICHUM SCLEROTIA FROM SOIL

LABORATORY EXPERIMENTS

The ability of *Phymatotrichum omnivorum* sclerotia to survive under different soil conditions in the laboratory was observed following the addition of known numbers of sclerotia to Houston and Hunt clays receiving different amendments. At intervals ranging from 10 days to 6 months, soil lots were examined for number of recoverable sclerotia and for microbial activity in the soil or on the sclerotial surfaces. Such studies showed that many more sclerotia were eliminated from soil containing fresh organic materials than from soil containing no such amendments.

When the data from five separate experiments, conducted during the course of 2 years, are considered collectively, the greater susceptibility of sclerotia in organic-amended soils is convincingly shown. In soil amended with barnyard manure or with chopped sorghum fodder, 64.9 and 69.7 percent of the sclerotia added were eliminated; in superphosphate-amended and in unamended soil, 14.4 and 19.6 percent, respectively, were eliminated. Other organic materials, such as cottonseed meal or hulls or chopped cotton roots, were found as effective as manure or sorghum fodder in reducing the sclerotial population in soil.

The results of a typical experiment are presented in table 5. The sclerotia used in this experiment were light brown-buff types washed out by hand from root rot-infested field soil; 95 percent of these sclerotia were found to be viable following field recovery. In other experiments analogous to the one recorded in table 5, the older dark-brown types of sclerotia recovered from field soil were employed; the

results obtained with such sclerotia were essentially the same insofar as contrast between check and organic-amended soils was concerned, although in several experiments there appeared a tendency for the old dark-brown types to be more resistant generally. Preliminary washings revealed that the Hunt clay employed was originally free of sclerotia; and also that the screening and washing methods employed permitted complete recovery immediately following the addition of sclerotia from either amended or unamended soil.

TABLE 5.—*Recovery of Phymatotrichum omnivorum sclerotia* [1] *from soil in relation to periods of incubation and types of soil fertilization*

Incubation period (days)	Sclerotia recovered from—				
	Unamended check	Soil fertilized with—			
		1 percent superphosphate	3 percent manure	3 percent sorghum fodder	3 percent chopped cotton roots
15	190	186	76	77	51
35	162	188	58	53	48
45	153	175	58	42	67
60	165	169	45	63	39
75	152	161	55	45	46
Total	822	879	292	280	251

[1] Number recovered after incubation periods indicated. 200 sclerotia were originally added to each container.

It was observed repeatedly that major reductions in sclerotial number were secured only during the periods of intensive microbial activity in soil. Since the microbiological data collected for the different laboratory experiments are extensive, such material is presented in summary form. Soil (in unamended or inorganic-amended lots) in which sclerotia persisted with relative impunity showed total micropopulations ranging from 15 to 50 million, expressed as per gram of air-dry soil, throughout the periods of incubation employed. Soil receiving barnyard manure or chopped sorghum fodder at 3-percent rates showed micropopulations of from 100 to 300 million during the first 2 weeks after treatment; following their initial precipitous increases, such micropopulations declined more gradually, until they were comparable to those in untreated soil. Inspection of table 5 shows that major reductions in sclerotial numbers were secured during the initial period of intense microbiological activity. Figure 2 shows the decomposed and hollow-shelled sclerotia after 15 days' burial in fertilized soil lots, and, in contrast, the undecomposed, solid-appearing sclerotia recoverable after burial in untreated soil.

Direct microscopical studies of stained Cholodny slides (*9*) showed general confirmation of the cultural data, and in addition, yielded some further information concerning microbial activity on or near the surfaces of buried sclerotia. After short burial periods in soil, numerous fungus strands were seen in the sclerotium-glass slide contact region; certain fungus strands appeared as *Phymatotrichum omnivorum* mycelia. After longer incubation periods, shadowlike strands of fungus mycelia covered with masses of bacteria were observed, suggesting early colonization of the sclerotial surface region by fungus strands, followed in turn by bacterial invasion and decomposition.

Spores of *Alternaria*, *Hormodendrum*, and *Trichoderma*, and long slender conidia of *Actinomyces* were observed; this suggested that mycelial stages of these forms accounted for some of the fungus strands observed shortly after burial of sclerotia in soil.

Germination of *Phymatotrichum omnivorum* sclerotia was suggested by the Cholodny slide studies. The possible role of germination of sclerotia in the differences in survivals in organic-amended and in unamended soil (table 5) was investigated in the following experiments.

Eight replicates of 5 soil conditions (untreated, 1 percent superphosphate, 3 percent chopped green alfalfa, straw, manure) were established in laboratory containers, and 10 sclerotia were buried in each of the 40 containers. Two lots of sclerotia were employed; one lot consisted of younger, light-brown sclerotia, of 80-percent viability; the second lot, of old, dark-brown sclerotia, of 50-percent viability. Aliquots of each lot were killed by heating to 85° C. for 5 minutes. In the preparation of the experiment, duplicate containers in each of 8 replicates received (1) untreated young sclerotia, (2) untreated old sclerotia, (3) killed young sclerotia, and (4) killed old sclerotia.

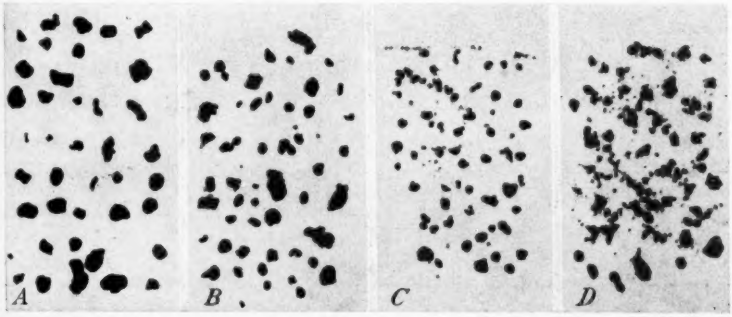

FIGURE 2.—Appearance of *Phymatotrichum omnivorum* sclerotia. *A*, Prior to burial in experimental soil lots. *B–D*, Following burial for 15 days in (*B*) unamended Hunt clay, (*C*) Hunt clay plus 3 percent chopped sorghum, and (*D*) Hunt clay plus 3 percent chopped alfalfa. About ×1.

All containers were incubated for 17 days and then examined for condition of the buried sclerotia. As all sclerotia were placed at known locations on glass slides, full recovery was possible. Observations were recorded on the basis of whether recovered sclerotia were solid and intact, or were disintegrating or hollow shells. A summary of observations is presented in table 6.

TABLE 6.—*Percentage of viable and killed sclerotia recovered from soil receiving different treatments*

Soil treatment	Young sclerotia		Old sclerotia	
	Living	Killed	Living	Killed
	Percent	*Percent*	*Percent*	*Percent*
Untreated (check)	20	5	15	0
Superphosphate	5	0	10	0
Ground straw	70	15	50	15
Ground alfalfa	75	30	40	25
Manure	75	40	15	5

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Field Observations

A field experiment provided four areas on root rot-infested land. Two areas received either barnyard manure or sorghum fodder thoroughly mixed into the upper 8 to 10 inches of soil by rotary plowing on October 3, 1939; a third area received similar deep tillage without organic amendment on the same date; and the fourth area received the usual bedding later in the fall season. All areas were cropped to cotton during the 1940 season.

Observations for the occurrence of sclerotia in these areas were made by field excavations during the latter part of August 1940. No sclerotia were found in the upper 12 inches of soil in areas receiving deep tillage or organic materials; sclerotia were present below the 12-inch level. In the normally bedded area, sclerotia were observed in abundance in the 6- to 12-inch level. The largest proportion of dead cotton plants occurred in this check area, and the smallest proportion in the manured area (table 7). Increased micropopulations were apparent in all the field areas after the first good fall rain in October 1939, but periods of prolonged microbial activity were noted only in areas receiving organic manures. Total micropopulations in the soil at five different dates of sampling, as well as dead cotton in the four areas at three different dates of mapping, are shown in table 7.

Table 7.—*Soil micropopulations and incidence of dead cotton in differently treated field areas*

TOTAL MICROBIAL NUMBER[1]

Dates of sampling or mapping	Plot treatment			
	Normal bedding	Deep rotary tillage	Deep tillage+barnyard manure	Deep tillage+sorghum fodder
	Millions	*Millions*	*Millions*	*Millions*
Oct. 13, 1939	159.5	335.5	452.6	287.9
Nov. 29, 1939	10.2	26.1	62.3	115.1
May 4, 1940	18.6	19.8	118.3	222.1
June 22, 1940	12.2	12.5	50.6	50.0
July 20, 1940	17.1	21.2	36.8	47.9

INCIDENCE OF DEAD COTTON PER PLOT[2]

	Percent	*Percent*	*Percent*	*Percent*
September 1939	85.3	80.0	96.0	74.0
August 1940	90.0	36.3	7.0	20.0
October 1940	94.0	69.0	34.0	60.0

[1] Expressed as millions per gram of air-dry soil.
[2] Expressed as percentage of total stand.

DISCUSSION

It is known that under field conditions *Phymatotrichum omnivorum* makes an extensive mycelial growth during the late summer and early fall; excavation studies during the fall season may reveal the parasite on the roots of many cotton plants even though no above-ground symptoms of the disease are evident. If the mycelial stage is recognized as capable of development upon a host-plant root system, and, in contrast to the saprophytic flora, of attacking healthy roots,

but if at the same time it is known to be incapable of successful competition with the large micropopulations engaged in the decomposition of fresh organic residues, the importance of ending the sheltered host-borne stage of the parasite as quickly as possible and thus prolonging the period during which it is subject to attack or competition of other soil micro-organisms becomes evident.

Cotton plants injured by late-summer clipping or girdling showed greatly intensified root-surface microfloras. Since the mycelium of *Phymatotrichum omnivorum* was inhibited or destroyed in organic-amended soils in which the increases in micropopulations over those of check soils not destructive to mycelium were of the order of 5 to 10 times, it is not improbable that the microbial activity on root surfaces, where the micropopulations increased as much as 100 to 200 times, provided an environmental factor unfavorable to the fungus. Growth of *P. omnivorum* from inocula on injured or decomposing cotton roots at practically any stage of decomposition, following sterilization to remove competitive microbial activity, further supports this reasoning. The limitation of survival of *P. omnivorum* mycelium on decomposing cotton roots is considered, therefore, more directly a matter of microbial interrelationships than of food exhaustion.

Phymatotrichum sclerotia, in contrast to the mycelium, appear very resistant to a prolonged soil-borne stage of existence; their longevity under fallow and different crop-rotation schemes is generally recognized. That sclerotia are susceptible to elimination from soil during periods of intensive microbial activity following applications of fresh organic residues is shown by the present work. The contrast between persistence of sclerotia in organic-amended and unamended soils within the first 10 to 15 days after burial was at first difficult to understand, and it was only after the Cholodny slide studies and the burial of heat-killed sclerotia that the increased germination of sclerotia under soil conditions favoring general microbial activity offered a partial explanation of the difference in survival. In the absence of a host plant, the emerging mycelium becomes exposed to saprophytic conditions, and the parallel results noted during the course of mycelium and sclerotia studies become understandable. It is not known why heat-killed sclerotia are more rapidly eliminated from organic-amended soil during the early period of incubation. It is possible that a mass-inoculation effect with more numerous and perhaps more diverse soil micro-organisms is obtained; it is also possible that the sclerotia substance presents a source of supply of one or more elements required during the initial decomposition stages of the added organic material by the soil microflora concerned.

The field observations on the absence of sclerotia in the organic-amended and tilled areas, together with the decreased incidence of dead cotton as shown by the August 1940 mapping, indicated that reduction in infectious material could be obtained in the field. However, it was not apparent from the work performed whether the more significant factor was (1) the elimination of root-borne mycelial strands important either directly as subsequent sources of infection or indirectly through their later production of sclerotia or (2) the elimination of soil-borne sclerotia formed prior to fertilization and tillage. If the former factor is the more significant, the destruction

of roots by early plowing becomes more important than the application of additional organic residues to soil. Clark and Thom (*4*) have expressed the opinion that any microbiological sanitation effects of added organic residues are accomplished through the agency of the soil microflora, since such fertilizations failed to affect the root microfloras of healthy cotton. If the destruction of soil-borne sclerotia is essential, the value of adequate mixing of fresh organic materials into soil to secure the greatest possible contact with soil-borne infectious material is apparent.

SUMMARY

Hunt clay to which no organic material was added permitted growth and persistence of *Phymatotrichum omnivorum* mycelium over the soil and root surfaces in open containers inoculated with segments of recently infected cotton roots, when maintained under favorable moisture and temperature conditions. Soil containing added organic matter, but otherwise similarly inoculated and maintained, either completely inhibited the growth of *P. omnivorum* or permitted initial growth which was followed by disintegration of mycelium.

Under field conditions, cotton roots injured during the late summer or early fall showed pronounced increases in micropopulations associated with root surfaces; such increases were proportionately greater than those caused in soil by organic amendments and found inimical to the growth of the parasite.

The majority of *Phymatotrichum omnivorum* sclerotia buried in organic-amended soil were quickly destroyed; in contrast, the great majority of sclerotia buried in soil without organic residues persisted. Sclerotia remaining after subsidence of the microbial activity occasioned by fertilization persisted with little further reduction in numbers for several months; for the elimination of either mycelium or sclerotia, treatments with organic materials were especially effective during the period of increased microbial activity.

Removal of the competitive effects of other microbes by soil sterilization, and subsequent reinoculation with *Phymatotrichum omnivorum*, permitted good growth of fungus mycelium, regardless of the amount of decomposition which the cotton roots or organic residues employed had previously undergone. Fungus survival was considered, therefore, to be limited by microbial interrelationships rather than by food exhaustion.

It was observed that during the early stages of incubation viable sclerotia disappeared more rapidly from amended soils than killed sclerotia; the importance of sclerotia germination as one factor in soil sanitation was thus suggested. That other factors were operative also was suggested by the greater elimination of heat-killed sclerotia from organic-amended soil than from unamended check soil.

Following field application of organic materials, together with early October plowing, increased microbial activity, reduction of the incidence of dead cotton in the succeeding crop, and greater difficulty of sclerotia recovery from the amended levels in field soil were observed.

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DISSEMINATION OF FUNGI THAT STAIN LOGS AND LUMBER [1]

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INTRODUCTION

In the literature on wood-staining fungi there are several descriptions (*2, 4, 5, 6, 7, 12, 13*) [3] of close associations of certain species of staining fungi with certain bark beetles and their tunnels, mostly in relation to stain in standing timber. There is, however, practically no general information on the means of dissemination of staining fungi to logs and lumber or the relative importance of these means. In fact, except for the work on bark beetle dissemination, most statements referring to dissemination of staining fungi seem to be based largely on supposition.

During the past 10 years considerable data on the dissemination of fungi that stain logs and lumber have been accumulated by the Division of Forest Pathology at its New Orleans branch. Brief accounts of some of these findings have been published (*9, 11*). Although the present studies are far from exhaustive they probably give an adequate picture of dissemination insofar as it bears on practical control of stain in logs and lumber in Louisiana and Mississippi. Although the study was limited to these two States, there is no reason to believe that the data would not be applicable to other States in the Southern Pine Belt.

MEANS OF DISSEMINATION

AIR CURRENTS

In an attempt to determine the prevalence of air-borne spores of staining fungi in mill yards, malt-agar plates were exposed for ¼, ½, 1, and 2 minutes, most often for 1 minute. In table 1 are listed the fungi secured in this manner. For convenience of comparison the results were transformed to the uniform basis of 125 plates exposed for 1 minute.

It is evident from table 1 that the number of air-borne spores of the important staining fungi in mill yards at a given time is not great but that the number of spores of some of the less important staining fungi is high. These results are based on exposures made in yards of average to good sanitation; none of the yards had severe staining of lumber at the time exposures were made. Because of the large

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[3] Italic numbers in parentheses refer to Literature Cited, p. 557.

TABLE 1.—*Fungi developing in malt-agar plates exposed in lumber-seasoning yards and inside mills*

Fungus	Fungi (calculated) per 125 plates exposed for 1 minute in—		
	Hardwood yards [1]	Pine yards [2]	Mills [3]
Important staining fungi:	*Number*	*Number*	*Number*
Ceratostomella ips Rumbold	0	0	4
Ceratostomella pluriannulata Hedgc	1	0	162
Endoconidiophora coerulescens Münch	2	0	15
Graphium rigidum (Pers. ex Fr.) Sacc	0	0	55
Helminthosporium spp	3	1	0
Minor staining fungi:			
Alternaria spp	19	7	7
Cladosporium spp	116	70	187
Pullularia spp	103	132	4
Torula spp	3	0	0
Dark, undetermined [4]	21	5	0
Nonstaining organisms	688+	980+	855+

[1] Based on 145 plates exposed at 4 mills on 11 different dates during 1937 and 1938.
[2] Based on 105 plates exposed at 4 mills on 7 different dates during 1937 and 1938.
[3] Based on 17 plates exposed in 2 mills on 4 different dates in December 1937. Mills were cutting mostly hardwoods at the times exposures were made.
[4] Some of the staining fungi listed may be included, but if so they were obscured by molds and bacteria

number of nonstaining fungi present, the agar-plate exposure method entailed excessive culture work in separating the developing colonies. Therefore this method was dropped in favor of exposures of sterile wood blocks.

Pine blocks approximately 1×1×5 inches were immersed for ½ minute in boiling water, and placed in sterile jars until exposed. At each of 2 mills, on 4 occasions, 20 of these blocks were suspended by wires attached to nails in the ends of the blocks. These mills were in central Mississippi and were approximately 50 miles apart. One mill had a clean yard with practically no stain; the other had a yard with a large pile of slabs at the edge that were green and there was heavy stain in much of the lumber. The blocks were exposed for 20 minutes and watched continuously for insect visitors. All blocks touched by insects were discarded. After exposure the blocks were

TABLE 2.—*Fungi developing on pinewood blocks exposed for 20 minutes in mill yards with light and heavy stain infection*

Fungi isolated	Blocks yielding fungi listed at indicated mill yard and date of exposure									
	Yard with light stain					Yard with heavy stain				
	May 11	June 23	July 28	Aug. 30	Total	May 11	June 23	July 28	Aug. 30	Total
Important staining fungi:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ceratostomella pilifera (Fr.) Wint	0	0	0	0	0	0	13	0	0	3
Ceratostomella ips Rumbold	0	0	0	0	0	0	0	14	0	3
Helminthosporium spp	18	0	36	7	14	0	6	21	22	10
Minor staining fungi:										
Alternaria spp	55	18	43	43	37	42	0	14	22	21
Cladosporium spp	91	6	36	7	30	79	0	36	11	36
Pullularia spp	45	18	0	7	16	16	0	0	33	10
Dark, undetermined [1]	0	12	29	21	16	11	13	14	44	17
Nonstaining organisms	91	100	100	79	93	95	100	100	100	98
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Blocks exposed	11	17	14	14	56	19	16	14	9	58

[1] Some of the staining fungi listed may be included, but if so they were obscured by molds and bacteria.

returned to their jars. On 3 of the 4 exposure days humidity was high. For each test 6 blocks were left unexposed as checks. Although molds occasionally developed on these checks, no staining fungi were found.

The fungi cultured from these blocks are listed in table 2. As in the case of agar-plate exposures, it is evident that the populations of spores of important staining fungi in the air were not great during the exposure periods, even in the yard with heavy stain and under humidity conditions favoring spore production and discharge. On other occasions *Diplodia natalensis* Evans and *Graphium rigidum* have been secured from the air, showing that all the important staining fungi of the Southern States (*10*), with the possible exception of *Diplodia* sp., are at times air-borne.

By far the most important staining fungi in the Southern States are *Endoconidiophora coerulescens* and *Ceratostomella pluriannulata* on hardwoods and *C. pilifera* and *C. ips* on pine. From the data presented it might seem at first glance that air dissemination of these species is of very little importance. However, it must be remembered that green wood in air-seasoning piles is in a condition favorable for infection over a period of days or weeks, depending on weather conditions. If the data are reexamined in the light of this fact, i. e., if the time were days or weeks, instead of minutes as given in the tables, it is evident that appreciable numbers of spores can settle on lumber in piles during the period of susceptibility to infection by staining fungi. Since there are marked convection currents in new seasoning piles, air-borne spores can be carried throughout the piles.

INSECTS

At 7 mills in Louisiana and Mississippi, on 20 occasions from 1937 to 1940, insects found on or in seasoning lumber or stored logs were collected in gelatin capsules, and, as soon as practicable, were transferred to malt agar. The fungi isolated from insects in this manner are listed in table 3.

In evaluating the importance, as stain disseminators, of the various insects listed in table 3, three factors must be considered: (1) The importance of the fungus species carried, (2) the habits of the insects, and (3) the abundance of the insects on green wood. Because of the first of these factors a line was added to table 3 giving the percentage of the various insects cultured that yielded important staining fungi. It will be noted that in the groups labeled "Other beetles," "Dipterous insects," and "Other insects," an appreciable number carried important staining fungi. However, individuals cultured in these groups were of species that are not primarily associated with wood but are chance visitors to lumber seasoning piles and ordinarily do not penetrate seasoning piles. Therefore they do not inoculate wood in the parts of piles where staining may be severe, and probably are of little importance in disseminating staining fungi to seasoning lumber. These insects probably become contaminated with spores from lumber ends stained prior to sawing and from logs.

The important insect disseminators of staining fungi are probably to be found among ambrosia beetles, bark beetles, powder-post beetles, and certain other insects whose life habits bring them in close contact with wood.

TABLE 3.—*Fungi isolated from insects collected in or on lumber* [1] *in seasoning yards*

Fungi isolated	Insects yielding fungi												
	Ambrosia beetles				Bark beetles			Miscellaneous insects					
	Platypus compositus	*Pterocyclon mali*	*Xyleborus affinis*	Undetermined	*Ips* spp.	*Orthotomicus caelatus*	*Pissodes* sp.	*Xylobiops basilaris*	Tenebrionids	Staphylinids	Other beetles	Dipterous insects	Ants	Other insects
Important staining fungi:	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
Ceratostomella ips	0	0	0	0	62	75	0	0	0	0	0	0	0	0
Ceratostomella pilifera	0	0	0	0	0	25	0	0	0	0	0	0	0	13
Ceratostomella pluriannulata	3	13	2	35	0	0	0	5	50	0	11	10	9	7
Diplodia natalensis	0	0	0	0	0	0	0	0	25	0	0	0	0	0
Endoconidiophora coerulescens	2	3	9	0	0	0	0	5	25	0	0	6	0	0
Graphium rigidum	5	10	0	0	3	0	0	45	75	45	6	3	5	27
Helminthosporium spp.	2	3	2	0	0	0	0	0	0	0	28	16	0	0
Total	10	27	11	35	64	100	0	55	100	45	39	32	9	33
Minor staining fungi:														
Alternaria spp.	0	0	0	0	5	0	27	10	0	0	17	13	9	0
Ceratostomella multiannulata Hedgc. and Davidson	0	0	0	0	0	0	0	0	0	0	0	6	0	0
Ceratostomella spp.	0	3	0	0	0	0	0	20	0	0	0	6	0	0
Cladosporium spp.	11	0	0	10	5	50	60	40	0	14	61	52	36	27
Endocomidiophora moniliformis (Hedgc.) Davidson	5	7	2	20	0	0	0	20	25	91	22	0	0	7
Pullularia spp.	8	7	0	0	5	50	47	10	0	5	33	29	0	27
Dark, undetermined [2]	7	3	6	5	13	25	13	5	0	0	17	13	5	13
Nonstaining organisms	93	93	100	100	100	100	100	90	50	100	94	100	82	100
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
Insects cultured	61	30	65	20	39	4	15	20	4	22	18	31	22	15

[1] Some of the ambrosia beetles were taken from galleries in newly infested logs.
[2] Some of the listed staining fungi may be included, but if so they were obscured by molds and bacteria.

AMBROSIA BEETLES

It has been shown (*9*) that in applying the common stain-control chemicals to stored hardwood logs, stain is not prevented if ambrosia beetle attack is heavy. Because of this it seems that ambrosia beetles must be common and effective carriers of staining fungi. Since ambrosia beetles heavily attack not only logs but also freshly sawed lumber of the most stain-susceptible hardwood species, such as sweetgum, they are also important in inoculating lumber. This inoculation is particularly effective, since the attacking adult bores deeply into the wood where the staining fungus would not be affected by any surface chemical treatment. The stain referred to in this discussion is not the restricted blackening around the tunnels of certain ambrosia beetles but the common stain found in sapwood of many tree species without beetle attack. Of the 176 adult ambrosia beetles cultured (*Platypus compositus* Say, *Xyleborus affinis* Eich., *Pterocyclon mali* Fitch, and undetermined ambrosia beetles), 16 percent yielded important staining fungi. Although this percentage is not high, it seems likely that ambrosia beetles are important stain disseminators since they attack both hardwood logs and lumber in large numbers.

Bark Beetles

The close associations of certain wood-staining fungi with *Ips* (*2*, *4*, *5*, *6*), *Dendroctonus* (*5*, *6*, *7*), and *Scolytus* (*12*, *13*) have been demonstrated. Most of this work concerns associations of fungi and beetles in standing timber, although some of it was done with felled timber (*4*).

During the present investigations the only important bark beetles encountered were *Ips*[4] and its close relative, *Orthotomicus caelatus* (Eich.). A high percentage of the adults of both genera cultured yielded *Ceratostomella ips*, and the probability is that all individuals carry this fungus. On one occasion fresh *Ips* engravings in a pine log that was as yet unstained yielded *Diplodia natalensis* and on another occasion *Diplodia* sp. Furthermore, from adult *Ips* collected in mill yards, *Graphium*, *Alternaria*, *Pullularia*, and *Cladosporium* were isolated; and from adult *O. caelatus* from mill yards, *Ceratostomella pilifera*, *Pullularia*, and *Cladosporium* were isolated in addition to *Ceratostomella ips*, which is the specific staining fungus associated with *Ips* spp. Both *Ips* and *Orthotomicus* are attracted in large numbers to fresh pine lumber in seasoning piles during the flight season and were observed penetrating deeply into the piles. When wanes are present, bark beetles penetrate through the bark and can thus inoculate below the chemical dips used for stain control. However, inoculation may also occur merely by spores left when the adults walk on the lumber surface. This is the reasonable explanation of the occurrence of *C. ips* in test lumber, previously reported (*10*). Probably the most important inoculations accomplished by bark beetles are in freshly cut pine logs, which are frequently attacked in large numbers when stored for only short periods. Bark beetle infestations in logs probably have increased with the present practice, in the South, of using dry log storage instead of storage in millponds as was previously common. During the present investigations most of the serious pine log stain observed was associated with *Ips* and *Orthotomicus* infestations.

When periodic outbreaks of *Dendroctonus frontalis* Zimm. occur in standing timber (*8*), it seems likely that this beetle is an important disseminating agent of *Ceratostomella pini* in mill yards as well as in trees and logs. There is also the possibility that the air may be full of spores from dead trees during active outbreaks, such as the recent one in eastern Virginia, near mills of the Camp Manufacturing Co. The close association of this beetle and the staining fungus *C. pini* has been demonstrated (*5*).

The only other bark beetle noted during this study was a species of *Pissodes* that was sometimes found tunneling into wanes on pine lumber. No important staining fungi, however, were isolated from the 15 adults collected on several occasions. This beetle was not abundant. There are other genera of bark beetles that attack green logs and probably carry staining fungi to some extent.

Powder-Post Beetles

No study was made of the *Lyctus* group, as these insects mature in wood too dry for stain development. However, one of the powder-

[4] Species not determined.

post beetles encountered, *Xylobiops basilaris* (Say), attacks green pecan, persimmon, and ash lumber. On one occasion 20 adults of *X. basilaris* were captured on or as they started to penetrate green pecan lumber, and 55 percent were found to be carrying important staining fungi. This high percentage was surprising, since at the mill where the beetles were captured they were observed only in pecan and ash woods, in which staining is not a problem, and since, so far as is known, *X. basilaris* requires at least 4 months to complete its life cycle. In 4 months most seasoning lumber would be too dry to support any luxuriant growth of staining fungi, and consequently emerging adults would not be heavily contaminated with such fungi. Little is known of the habits of this beetle, but it is possible that after emergence the adults from which the cultures were made had visited stained sweetgum or other lumber bearing a heavy growth of staining fungi, or that the adults had emerged from logs where the moisture content remained sufficiently high during the life cycle of the insect for abundant fruiting to occur in the tunnels at the time of emergence. Only if it can be shown that *X. basilaris* commonly visits the more stain-susceptible species of wood in its journey to the wood species in which it breeds can it be considered an important agent of dissemination. The number of this beetle observed was not large.

Miscellaneous Insects of Possible Importance

When lumber or logs support growths of staining or molding fungi on the surface, there are commonly present large numbers of adults of staphylinids, a small predaceous carabid, and tenebrionids, and also a small dipterous larva. These insects wallow in the fungus growth and undoubtedly become covered with the spores of the fungi fruiting on the wood surface. Apparently little is known of the habits of these insects and the extent to which they migrate to fresh unstained wood. The staphylinids and tenebrionids, however, have been observed in appreciable numbers in bulk-piled green lumber that was waiting to be stacked in seasoning piles. The values in table 3 for these insects are based on isolations from adults captured on bulked lumber.

Mites

Mites are common in lumber-seasoning piles. Although no isolations were attempted from them it is conceivable that they cause considerable secondary spread of staining fungi within a given pile. Mites occurring under bark, boards, slabs, etc., are known often to carry spores (*3*).

Milling Machinery

Lumber may be heavily inoculated during the milling operation. A certain amount of fruiting of staining fungi commonly occurs on logs, particularly at the ends of hardwood logs. Inoculum from the ends of logs is thrown into the air by saws and also carried by saws and rollers to the surface of lumber coming in contact with them. In table 1 are listed the results of exposure of agar plates inside mills. The two mills in which exposures were made were cutting mostly hardwoods at the time the tests were made. The log supplies were average—not heavily stained. It is evident from table 1 that spore populations inside mills are large as compared with those in seasoning yards.

That untreated wood leaves mills inoculated with spores of staining fungi was further demonstrated by incubating in sterile jars pieces of bright wood collected at the edger and end trimmer. Of 25 pieces collected during the summer and winter of 1937, 20 stained heavily with *Endoconidiophora coerulescens*, *Ceratostomella pluriannulata*, and *Graphium rigidum* on hardwoods, and *C. ips* and *C. pilifera* on pine. When only logs apparently free of stain were being cut, about one-half the blocks of both pine and hardwood remained stain-free, but when stained logs were being cut all the blocks stained heavily even though the test material was taken several feet from visible stain or from stain-free logs.

RAIN WATER

Staining fungi have been caught in rain water dripping from lumber piles. In a properly constructed pile, water-carried spores are probably of little importance since rain water merely runs over the outside of the pile, inoculating wood that usually dries too rapidly for much stain development.

INFECTED WOOD

Dissemination by the transport of infected wood probably has a practical bearing only in the case of stained green cross stickers used in seasoning-pile construction. At some small mills, untreated green edgings are used for cross stickers and frequently are infected before they are used in the pile. "Sticker stain," resulting from the direct transfer of the staining fungus from the sticker to the lumber in contact with it, is common in these cases. Transfer from stained to unstained wood by direct contact has been experimentally secured with *Ceratostomella ips*, *C. pilifera*, *Diplodia natalensis*, and *Endoconidiophora coerulescens*. Other fungi were not tried.

RELATION OF CHEMICAL CONTROL TREATMENTS TO DISSEMINATION

It is now a common practice to dip freshly sawed lumber in a toxic chemical solution prior to air seasoning (*9*). Undoubtedly this practice has markedly changed the importance of some means of dissemination. Fungus spores disseminated during the milling operation are supposedly killed in passing through chemical dips. Likewise, air-borne spores and those carried by insects lighting and crawling on treated lumber are probably rendered largely ineffective by any toxic coating on the surface of the lumber. During prolonged periods of wet weather, when chemical treatments may fail to protect wood from staining, spores carried by air currents probably still play an important part in the dissemination of staining fungi. Since chemical treatment of exposed wood on log ends is the exception rather than the rule, dissemination to these ends by means of air currents and nonpenetrating insects is still relatively important.

Chemical dips have affected not only the importance of primary inoculations in fresh lumber but also secondary spread within seasoning piles by reducing the amount of inoculum present. At 28° to 30° C. the time between inoculation and production of new spores on untreated green wood was 46 hours for *Ceratostomella ips*, 22 hours for *C. pilifera*, 22 to 70 hours for *C. pluriannulata*, 22 to 46 hours for *Endoconidiophora coerulescens*, and 22 hours for *Graphium rigidum*.

These spores, resulting from primary infections, can be carried by various means to other parts of the same lumber pile before the surface of the wood is too dry for infection. Although similar values are not available for sporulation on treated wood, it can be assumed that most primary inoculations would never cause infections and that where infection occurs the period before new spore production would be longer than on untreated wood, resulting in much less secondary spread within the pile.

From observations made during the testing of commercial stain-control chemicals (*9*) it was obvious that these chemicals had little effect as insecticides against ambrosia beetles. Although based on less evidence, there are indications that stain-control chemicals likewise have little deterrent action on bark beetles boring into bark fragments left on treated lumber or on beetles crawling on treated lumber. Thus the chemical treatments probably have had little effect on spores carried by ambrosia and bark beetles where inoculation is made below the treated surface. Treatment, however, would reduce the importance of inoculations made by these beetles on the wood surface where they light and crawl prior to penetration.

DISCUSSION AND SUMMARY

Staining fungi are disseminated by means of air currents, insects, milling machinery, rain water, and the transport of infected wood.

Of the important staining fungi, *Ceratostomella pilifera*, *C. ips*, *C. pluriannulata*, *Endoconidiophora coerulescens*, *Graphium rigidum*, and *Diplodia natalensis* were found to be carried by air and insects; *C. pilifera*, *C. ips*, *C. pluriannulata*, *E. coerulescens*, and *G. rigidum* by milling machinery; and *C. pilifera*, *C. ips*, *E. coerulescens*, and *D. natalensis* by the transport of infected wood. Although most staining fungi are disseminated by various means, *C. ips* and probably *C. pini* are disseminated mostly by the bark beetles with which they are specifically associated.

Air-borne spores are now of less importance than before chemical treatments were in common use. However, they probably remain important since they may infect log ends, untreated lumber at the few mills not employing dips, and treated lumber during prolonged wet periods, when chemical treatments are not particularly effective.

Although many species of insects commonly carry staining fungi, the effectiveness of inoculations by most species is lessened by the fact that inoculations are in the outer parts of seasoning piles, where the lumber dries too rapidly for much stain development, and by the use of chemical treatments. However, two groups of insects, the ambrosia beetles and the bark beetles, remain of practical importance as carriers of staining fungi. Bark beetles are important mainly in inoculating pine logs, and ambrosia beetles in inoculating hardwood logs. Ambrosia beetles also attack green lumber of species most susceptible to staining, inoculating it below protective surface chemicals. All the indications are that none of the commercial stain-control chemicals in common use have any repellent effect on ambrosia and bark beetles. For the control of stain there is need of cheap and effective methods of repelling ambrosia and bark beetles

from logs and the former also from lumber. A start has been made toward filling this need (*1*).

Dissemination and inoculation during the milling process are important when no chemical treatment is applied to the lumber as it leaves the mill. This importance increases when stained logs are being milled. Since chemical treatments are now commonly used to reduce stain infection, spores disseminated within mills are probably of little importance.

Spores carried by rain water probably are not of much importance, since in well-constructed seasoning piles water does not penetrate the interior of the piles, where most serious staining occurs.

Dissemination by the transport of infected wood may be important in causing "sticker stain," when stained green cross stickers are used in constructing seasoning piles.

In the practical application of data on dissemination, it is necessary to consider not only the agents of dissemination but also the sources of inoculum. It was pointed out that the general use of stain-control chemicals has probably greatly reduced the amount of inoculum of staining fungi in seasoning yards. Previously reported observations (*11*) indicate that slabs, edgings, and similar green material are suited to the production of spores of staining fungi in large numbers but that old and weathered debris is not. Therefore, sanitation in and around seasoning yards should include restriction of accumulations of green refuse of both pine and hardwood. With hardwoods, fruiting of staining fungi is also common on the ends of logs and the ends of lumber cut from stained logs (*10*). This argues for quicker utilization of hardwood logs and wider use of chemical treatments for hardwood log ends (*9*).

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